Clinical and epidemiological studies from a tuberculosis referral center in The Netherlands

Cécile Magis-Escurra

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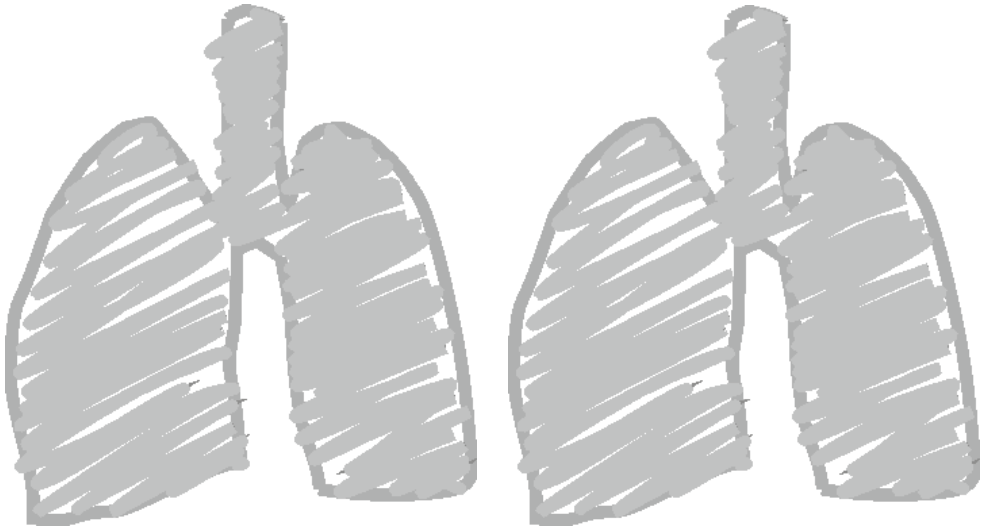
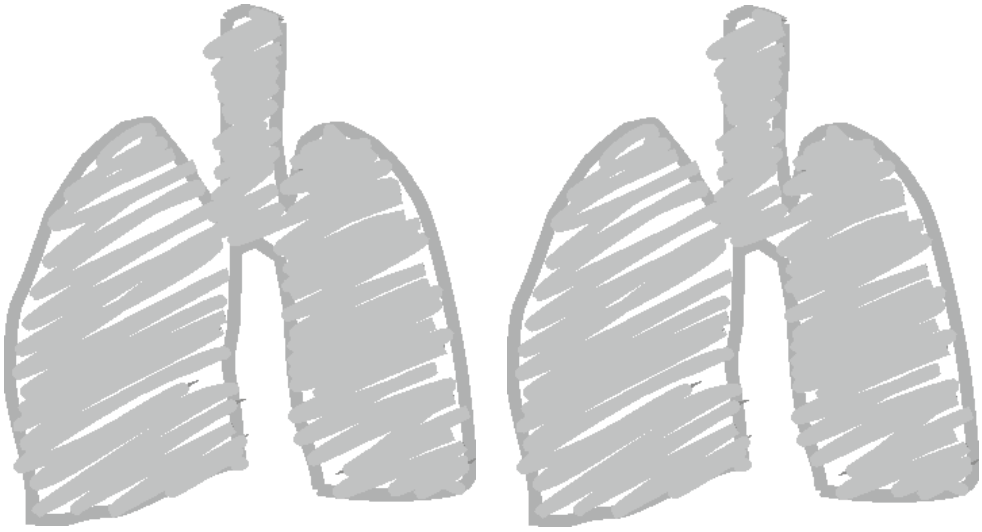


Chapter 1

General introduction and outline thesis



General Introduction

Tuberculosis (TB) is a major public health problem and the second killer worldwide due to a single infectious agent. It is caused by bacteria from the *Mycobacterium tuberculosis* complex (*tuberculosis, africanum, bovis, microtii, canetii* and *bovis BCG*). More than 80% of all TB patients live in sub–Saharan Africa and Asia. In 2010, a worldwide incidence of 8.8 million individuals with active TB were registered and an estimated 1.4 million people died. The incidence of active TB is slowly declining and the TB death rate dropped 40% between 1990 and 2010.[1]

Many countries worldwide, however, increasingly struggle with Non-Tuberculous Mycobacteria (NTM), especially those countries with declining incidence of TB.[2,3] Next to *M. tuberculosis* complex, NTM are the second disease entity within the genus Mycobacterium. Other clinically relevant mycobacteria are *Mycobacterium leprae* which causes leprosy and *M. ulcerans*, causing Buruli ulcer.

This introduction discusses general, diagnostic and treatment principles of TB and NTM diseases. We subsequently shift our focus to the Dutch situation for both disease entities.

Tuberculosis

A historical perspective and pathogenesis

TB is an ancient disease, as has been demonstrated by studies in which mycobacterial DNA was isolated from mummies (±2050-500 B.C) by spoligotyping.[4] The TB discovered in early mummies is a bovine form, proving TB has accompanied mankind since it started domesticating cattle. In 1882 Robert Koch identified the main causative organism, *Mycobacterium tuberculosis*. In TB, bacteria are spread by airborne micro-droplets from an infectious patient. Transmission occurs when a second person inhales these infected droplets. After inhalation, the bacteria are phagocytosed by macrophages in the alveoli. Provided there is an adequate immune response, the bacteria are subsequently encapsulated and remain in the body in a low metabolic, dormant state (latent tuberculosis infection). Progression to active disease occurs in 5-10% of infected individuals. In 50% of these progressive cases, the disease develops within the first two years after infection (primary TB). Primary disease is most likely to occur in infants and children before 5 years of age, patients with advanced HIV infection or those using tumor necrosis factor (TNF) antagonists. Yet the remainder flares up years and years later when immune status is impaired due to ageing, malignant disease, immune suppressant drugs and other conditions (secondary or reactivated TB).

Clinical presentations

TB has many appearances and may affect every organ of the human body, except hair and nails. The lungs are not only the portal of entry for M. tuberculosis, but they are the organ most likely involved clinically by the microbe (>50% of cases).[5] Patients with TB may display widely variable symptoms. Typical respiratory complaints are productive cough, chest pain, hemoptysis, shortness of breath and hoarseness. Constitutional symptoms consists of malaise, weakness, fever, night sweats and anorexia. Some patients, mainly the patients with pulmonary TB or disseminated TB, can be extremely ill, whereas others with paucibacillary TB tend to have only mild symptoms.

Diagnosis

Until now a definite diagnosis of TB is only made by the gold standard (positive culture of *Mycobacterium tuberculosis* complex). The (sum of) Tuberculin Skin Test (TST), interferon gamma release assays (IGRA), Ziehl Neelsen staining of acid fast bacilli and Polymerase Chain Reaction (PCR) of *M. tuberculosis* complex give an indication in the direction of TB but are only supportive to the diagnosis. In case of pulmonary TB with large cavities, culturing *M. tuberculosis* complex is not too difficult. In extrapulmonary or pediatric TB the diagnosis already becomes more challenging. Furthermore, diagnosing TB in a HIV positive patient with a low CD4 leukocyte count can be quite problematic at times.

For a long time, the World Health Organization (WHO) propagated diagnosing tuberculosis in endemic regions with smear microscopy and (if available) radiography. In the era of raising incidences of drug resistant TB worldwide this is no longer sufficient, as we need the mycobacterial strain from culture to perform drug susceptibility testing.

Knowing that in 2009 only 12% of the estimated 250,000 prevalent multi drug resistant TB cases could be identified, we may call this WHO policy outdated now.[6] Internationally, especially in endemic regions, there is an urgent need for mycobacterial culturing facilities and adequate trained personnel. The recent introduction of the GeneXpert® in 2010 was a big step towards possible fast and accurate diagnosis of probable drug resistant tuberculosis, yet the high price of this PCR-based diagnostic method is still an issue to overcome, especially for the resource-limited settings where drug resistant TB is most prevalent.

Treatment of tuberculosis: history, current treatment and optimization of treatment

Improved socio-economic conditions during the industrialization period in the last century lowered the incidence of tuberculosis. Effective treatment, however, did not exist until the forties of the previous century. Resting, adequate nutrition and fresh air in TB sanatoria could actually cure a quarter of the TB patients, another 25% developed a chronic form of TB and the rest died of TB. Collapsing the lung in order to cure tuberculosis was the last major thrust of clinicians before the modern era of chemotherapy. The first antituberculosis drugs on the market were streptomycin and para aminosalicylic acid (PAS).[7]

Soon after the introduction of these drugs it became evident that monotherapy was readily inducing drug resistance and the concept of combined drug therapy for TB was born.[8]

From 1952 isoniazid was introduced and combination therapy of streptomycin in the first 3-6 months together with isoniazid and PAS resulted in cure rates of 90-95%. Unfortunately, it required up to 24 months of continuous treatment to achieve this goal.[7,9] With the introduction of rifampicin and pyrazinamide, success rates of 95-97% were reached in the registration studies[7,9] and the shortened treatment duration from 12 to 6 months in 1993 was considered a big leap forward.

Although this treatment regimen is theoretically still highly effective, in practice global treatment success rates are only 86% on average.[6] Current TB treatment needs optimization as its long duration, adverse events and suboptimal pharmacokinetics lead to treatment failures, relapses and emergence of drug resistance and as a result: ongoing transmission in practice.[10]

From a classical clinical pharmacological perspective, pharmacokinetics and pharmacodynamics determine the eventual effect of TB drugs. The relation between the actual drug dose taken and the (time course of) plasma drug concentrations achieved is described by the pharmacokinetics of the drugs.

The plasma concentrations are related to treatment outcome (including problems of suboptimal response, emergence of resistance and adverse events) as determined by pharmacodynamics. Therefore, plasma drug concentrations are an important intermediary link between the dose administered and the desirable and undesirable effects.

From the schematic interplay between adherence, pharmacokinetics and pharmacodynamics in figure 1 it can be easily understood that factors such as suboptimal treatment regimens, non adherence, low plasma drug concentrations and resulting drug resistance, alone or in a combination, form threats for effective treatment.

Other co-factors influencing treatment outcome are for example the localization and extensiveness of disease or HIV infection. On itself, HIV infection also raises the lifelong incidence rates of TB. Drug resistance, merely a manmade problem, is a result of the administration of inadequate regimens and/or non-adherence to therapy together with the spontaneous

mutation capacity of mycobacteria during treatment.[11] Iseman referred so aptly to the development of drug resistance by his article titled: "Extensively drug-resistant Mycobacterium tuberculosis: Charles Darwin would understand".[12]

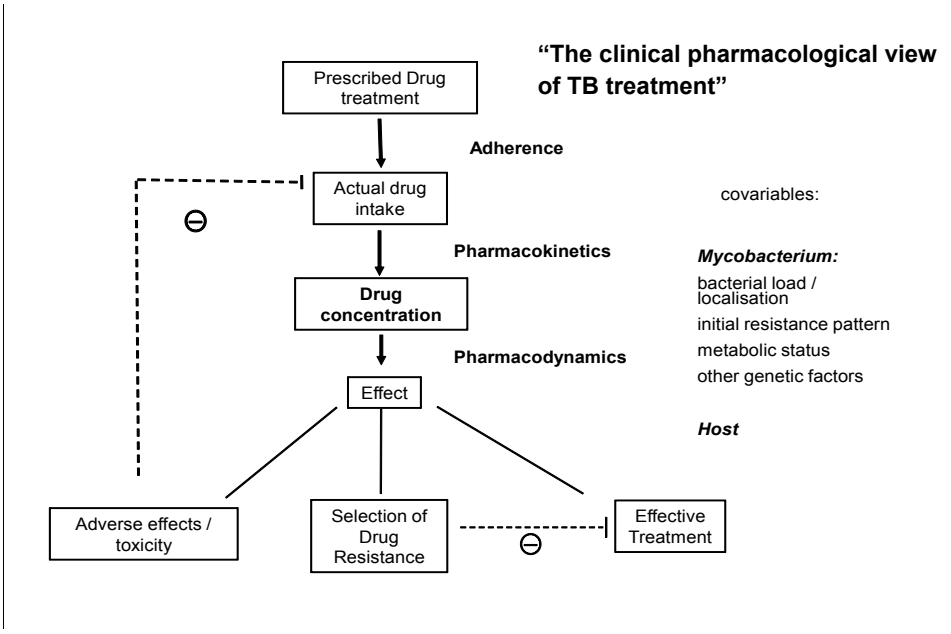


Figure 1.

From this schematic interplay we can also understand that shortening treatment regimens may be a key to improve treatment success rates.[13] Shortening TB treatment by optimizing rifampicin pharmacokinetics resulting in faster culture conversion to shorten TB treatment has been propagated since 2003[11,14-16] and is currently subject of clinical trials. With rifampicin we seem to be at the lower end of the dose response curve and we also know from pharmacokinetic studies in the last two decades that many TB patients have low serum concentrations.

At the introduction of rifampicin to the market issues as high costs, feared toxicity and the urgent need for an effective sterilizing regimen led to the choice of 600 mg of rifampicin.[14,16] Unfortunately, data regarding both the pharmacokinetics and pharmacodynamics of TB drugs are limited. Clinical trials of higher dosages of rifampicin are currently underway but to perform dose finding studies first, then tolerability and toxicity studies and lastly large phase III trials it will take quite some time before appropriate proof will be available. [clinical trials.gov Identifier: NCT01392911 /NC T00760149].

As illustrated above diagnostics and treatment both need to be optimized and much work has to be done to achieve the Stop TB partnership goal of reducing the global incidence of active tuberculosis to less than one per million population per year in the year of 2050.[17] However, tuberculosis research is complicated due to long duration of treatment, the lack of surrogate markers for response, a follow up relapse detection period of at least one year and the lack of well equipped research sites in endemic regions.

The current problem of rapidly expanding multi-drug-resistant tuberculosis understandably distracts researchers' attention from drug susceptible TB to the development of new drugs for drug resistant TB. However, limiting TB transmission by offering optimized first line therapy schemes in terms of treatment duration might prove to be more effective to control the epidemic.[18]

Non-Tuberculous Mycobacterial Pulmonary diseases

A historical perspective and pathogenesis

In the last decades, the incidence of NTM infections is increasing worldwide due to factors including improved detection methods, advancing age, increasing incidence of COPD, widespread use of antibiotics and immunomodulating drugs, and greater awareness of clinicians that these pathogens are able to cause serious disease in humans.[2,19-20]

After the discovery of the main culprit in tuberculosis pathogenesis, 'mycobacteria other than M. tuberculosis' were subsequently discovered from animals and the environment, especially in water and soil.[21] Likewise, these mycobacteria can be detected microscopically with Ziehl Neelsen and auramine staining as well as TB. Macroscopically, they exhibit different growth patterns from *M. tuberculosis* complex. NTM were first classified in 1959 by Runyon, into four groups on the basis of growth rates and pigmentation under the influence of light.[22] This classification is summarized in table 1.

The route of infection is by ingestion (food) or inhalation (shower). NTM infections are not transmissible from person to person. Dormancy, as in TB, is not known for NTM infections.

Table 1. Classification of Non Tuberculous Mycobacteria

Group	Characteristics	Clinically most important species
I	Slow growth; pigmentation after exposure to light	M. kansasii, M. szulgai, M. simiae
II	Slow growth; pigmentation without light exposure	M. xenopi, M.gordonae, M. scrofulaceum
III	Slow growth; no pigmentation	M. avium, M.intracellulare, M. malmoense
IV	Rapid growers	M. abcessus, M. fortuitum, M.chelonae

Clinical presentations

With this classification microbiology laboratories were now able to identify different species of NTM and this resulted in the characterization and distinction of clinically relevant diseases. Manten was the first to focus on the issue of clinical relevance of NTM in The Netherlands in 1965.[23] TB resembling pulmonary NTM disease by *M. kansasii*, cervical lymphadenitis in children by *M. scrofulaceum*, skin disease by *M. marinum* (fish tank granuloma) were commonly found diseases.[2] Either systemic (HIV infection) or local (bronchiectasis, coal miners lung) defects of the immune system are facilitating the growth of NTM's causing clinically relevant disease.

Pulmonary NTM disease can present radiologically with cavities, nodules and bronchiectasis but also as a hypersensitivity pneumonitis with diffuse infiltrates and nodules and a mosaic pattern on CT scan.[24-25] The clinical course of NTM is generally more prolonged than in TB and patients present with cough, fatigue, fever and weight loss, depending on the underlying lung disease and NTM species. Extra pulmonary NTM disease is rare, except for lymphadenitis caused by *M. scrofulaceum* or *M. avium* in children below 6 years of age.[26] Dissiminated extra pulmonary NTM disease generally affects immunocompromised patients (HIV or after organ transplant). The above described NTM disease is mainly caused by slowly growing NTM. Rapid growers (especially *M. abcessus*) cause difficult to treat eye and ear disease as well as pulmonary disease in patients with cystic fibrosis.[27-30]

Diagnosis

Contrary to TB, it is possible to encounter NTM bacilli from sputum (coincidental presence in the respiratory tract or due to contamination of the sample during collection) on one or more occasions without clinical significance.[2] This makes positive NTM cultures difficult to interpret for many clinicians. To diagnose clinically relevant NTM disease it is necessary to collect clinical data, perform radiological tests, grow microbiological cultures and subsequently have the NTM strain determined.

To differentiate clinically relevant from non clinically relevant NTM disease, the American Thoracic Society (ATS) has published criteria to aid in this diagnostic process.[2] These criteria are a combination of clinical and radiological features together with microbiological data. In The Netherlands, these criteria are increasingly adopted by clinicians.

Table 2: Summary of the American Thoracic Society diagnostic criteria[2] for pulmonary NTM infections

Clinical
1. Pulmonary symptoms, nodular or cavitary disease on chest radiograph, or an HRCT scan that shows multi focal bronchiectasis with multiple small nodules. And
2. Appropriate exclusion of other diagnoses.
Microbiological
1. Positive culture results from at least two separate expectorated sputum samples.
2. Positive culture results from at least one bronchial wash or lavage.
3. Transbronchial or other lung biopsy with mycobacterial histopathologic features and positive culture for NTM or biopsy showing mycobacterial histhopathologic features and one or more sputum or bronchial washings that are culture positive for NTM.
4. Expert consultation should be obtained when NTM are recovered that are either infrequently encountered or that usually represent environmental contamination.
5. Patients who are suspected of having NTM lung disease but who do not meet the diagnostic criteria should be followed until the diagnosis is firmly established or excluded.
6. Making the diagnosis of NTM lung disease does not, per se, necessitate the institution of therapy, which is a decision based on potential risks and benefits of therapy for individual patients.

Patients who meet these criteria are likely to have true pulmonary NTM disease; their NTM isolates can be considered clinically relevant.

Treatment of NTM disease: history, current treatment and optimization of treatment

In the first years after the discovery of the NTM as causative organism of disease it was thought that surgery was the only treatment option.[23] Observational studies of NTM disease treated with drugs formed the basis for the few randomized controlled trials conducted so far. The first prospective trial of treatment of *M. kansasii* infections was published in 1994 by the British Thoracic Society (BTS).[31]

In the same period, studies of macrolide based multi-drug therapy for *M. avium* infections were published. Together with the experience from dissiminated *M. avium* infections in HIV infected patients we learned that NTM disease is curable with multidrug regimens consisting of rifamycins, ethambutol and macrolides[33] although consensus about the most favourable treatment regimen is still not reached due to conflicting results of important randomized controlled trials. Especially the benefit of macrolides for treatment of pulmonary NTM disease in non-HIV infected individuals remains controversial.[2,33]

Further studies are needed, especially in NTM disease caused by species other than *M. avium* complex and *M. kansasii*, which are mostly even harder to treat.[27,34-35]

In TB the sequence of adherence to drug treatment, pharmacokinetics and pharmacodynamics has been subject to several studies so far. In NTM disease there are only a few, though important, publications from Wallace[36] and Peloquin[37] which unfortunately never resulted in a reevaluation of dosing strategies until now.

Considering the available evidence so far we feel it calls for optimized dosing regimens in NTM disease as we understand from the studies in TB patients that suboptimal plasma concentrations may lead to treatment failure and relapses.[11] Considering all the above it always remains important to emphasize that the decision to institute drug therapy in NTM disease should be based on the evaluation of potential risks and benefits for the individual.[2]

TB referral centers in The Netherlands

From the introduction of effective drugs along with an excellent public health protocol of contact tracing and screening of immigrants, The Netherlands has decreased incidences of tuberculosis to an average of 6.5 : 100,000 person/year in 2010[3][8] Only Scandinavian countries and the United States of America have comparatively low incidences.
In native Dutch individuals, the incidence is even lower: immigrants show much higher incidences of tuberculosis. In The Netherlands, 1000 cases of active tuberculosis emerge every year, of which 50% are diagnosed by pulmonary diseases specialists, 25% by the public health TB doctors and the rest by infectious disease specialists and others. The low incidence undoubtedly is a very exciting development. Yet this declining incidence also means progressively less exposure of doctors to tuberculosis, leading to more doctor's delay, more difficulties in diagnosing TB (especially in smear negative and extrapulmonary TB) and subsequently increasing morbidity and possibly higher transmission rates in the community.[39]
For this reason, the Dutch tuberculosis foundation (KNCV) has introduced the National Tuberculosis Consultants in 1983. These pulmonary disease specialists can be consulted by colleagues in case of difficulties in diagnosing or treating tuberculosis. The four consultants work in the two national referral centers for tuberculosis (Dekkerswald, Groesbeek/ Beatrixoord, Haren) where complex tuberculosis patients can be hospitalized for treatment or in case of necessity of (forced) isolation.

In practice, individuals with HIV infection, multi or extensive drug resistant tuberculosis, toxic drug reactions, severe co-morbidity, substance abuse, psychiatric disorders or homelessness are hospitalized for some time during treatment. Also patients with complex NTM disease are increasingly hospitalized in these TB referral centers as their prevalence among the largest patient population of a respiratory disease specialist (i.e. COPD patients) is increasing and clinically relevant disease necessitating drug treatment may lead to serious morbidity. In general, hospitalization rates in both referral centers have increased significantly in the last few years (rapport KNCV 2010).
Nowadays, around 200 patients are hospitalized in a referral centre each year. Having all possible study techniques and of course the financial means to offer patients the most optimal treatment under supervised circumstances we are able to perform epidemiological, diagnostic and pharmacological studies in these institutes at an academic level.

Aims and outline of this thesis

This thesis covers a range of subjects related to TB and NTM disease. By addressing epidemiological, diagnostic and pharmacological issues both in tuberculosis and NTM lung disease, we provide an overview of activities in a tuberculosis referral centre in The Netherlands.
Chapter 2 and 3 are devoted to epidemiological aspects of TB and clinical characteristics of the patients involved. Chapter 2 describes the population of TB referral centre Dekkerswald in the period 2000-2005. After this study we hospitalized, in a short period, three female native Dutch immunocompromised patients with TB caused by *M. bovis* in Dekkerswald. We became interested in the current prevalence and characteristics of *M. bovis* TB.
Chapter 3 describes these cases together with the current epidemiology and characteristics of patients with *M. bovis* in The Netherlands.

Chapter 4 has its focus on TB diagnostics. With data provided from the Netherlands Tuberculosis Registry we investigated all cases from 2007-2009 that were not culture confirmed. We investigated how and where diagnosis was made, why culture result was negative or why culture was not performed. We classified these non-culture confirmed diagnoses in 'no', 'possible', 'probable', and 'confirmed' TB, according to the European Centre of prevention and Disease Control (ECDC) TB case definitions for the accuracy of the diagnoses.

Chapter 5, also focusing on diagnostics, is a study of spinal TB diagnoses. We investigated whether a decreasing TB incidence leads to increase of diagnostic delays in The Netherlands in the period 2000 to 2011. We studied patient records at the public municipal TB services and compared results with data provided from the Netherlands Tuberculosis Registry. We describe current epidemiology and characteristics of patients with spinal TB.

After diagnostics we switched to pharmacokinetics of TB and NTM treatment. **Chapter 6** describes the pharmacokinetics of TB patients admitted to tertiary referral TB centers in The Netherlands and compares results with data from the literature. We assessed with linear regression analysis the sampling points correlating best with the area under the concentration versus time curve (exposure). This may enable more convenient yet sufficiently accurate estimation of exposure in future drug trials and in patient care. **Chapter 7** focuses on the pharmacokinetics of patients in Dekkerswald suffering from a recurrent TB.
These patients all suffered from a cavitary TB and showed absorption problems due to alcohol abuse, gastric surgery in the past and diabetes mellitus. We performed dose adjustments based on plasma concentration measurements (Therapeutic Drug Monitoring) and evaluated its effect on treatment outcome and adverse events. In **chapter 8**, the pharmacokinetics of drugs in NTM pulmonary disease were evaluated.
We studied the interactions between rifampicin, ethambutol, isoniazid, moxifloxacin, azithromycin and clarithromycin.
Finally, in **chapter 9**, the summary and general discussion, the results of the studies described are summarized. Subsequently the implications from our studies for future TB care and prevention, as well as possible future research directions are discussed.

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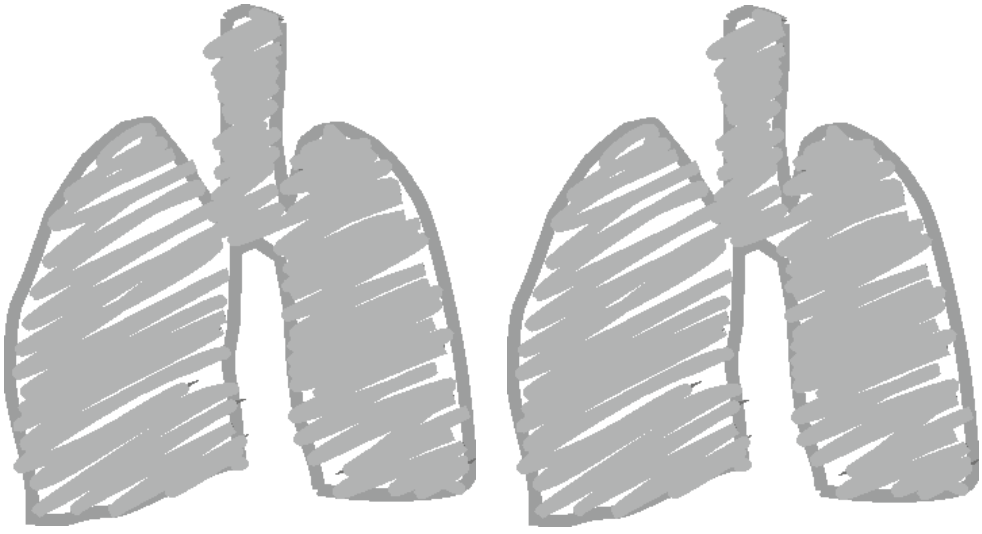
Chapter 2

Characteristics and treatment of tuberculosis patients in Dekkerswald 2000-2005



Abstract

Objective: to describe the patient population in Dekkerswald, Nijmegen, one of two tuberculosis (TB) centres in The Netherlands.

Design: descriptive, retrospective study.

Method: examination of medical records for all TB patients hospitalized between 2000 and 2005, including demographic, social, clinical and follow-up data.

Results: data from 166 patients were analysed. Tertiary referrals accounted for 98% of all hospitalisations. Most patients (68%) were referred for clinical reasons, and 32% were referred for social reasons. Drug resistance was encountered in 23% of patients; 9% had multidrug-resistant TB. Ten percent of patients were seropositive for HIV. Toxicity and side effects of treatment often led to changes in treatment (40%). Patients had pulmonary TB (59%), extrapulmonary TB (23%) or both (17%). Overall, 141 patients (85%) completed treatment. The TB-related mortality rate was 5%.

Conclusion: in Dekkerswald, there is a selected patient population that is characterised by drug-resistance, comorbidity, side-effects, extrapulmonary disease and social issues. Due to the low prevalence of TB in The Netherlands, knowledge and experience regarding complex types of TB are limited. Centralisation of patient care is important to preserve and optimise this expertise

Introduction

Tuberculosis (TB) is an infectious disease and until the mid-1980s its incidence in the Netherlands gradually decreased and was mainly limited to risk groups. Since 1987 the incidence increased as a consequence of immigration.[1] This effect has also been observed in other European countries.[2-5] Other determinants of the rising incidence, which persisted until 1994, were international travel for work and tourism and HIV infections.[6-7] After 1994 the number of new cases fell to 8 per 100,000 inhabitants (about 1100 cases) per year.[1,6]
In The Netherlands, TB patients are mostly treated by a pulmonologist (52%), a public health physician active in the area of tuberculosis control (24%), or an internal medicine specialist (12%).[1] Patients with a complex form of TB or with complications in the disease progression are often referred to a centre. In the practice guideline 'Medical treatment of tuberculosis 2005' contacting a tuberculosis expert is advised for certain forms of TB.[8]
The University Centre for Chronic Diseases Dekkerswald is part of University Medical Centre Nijmegen. Dekkerswald is one of the two TB centres still active in The Netherlands; the other is Beatrixoord in Haren.
Dekkerswald also has clinical and outpatients departments for general pulmonary diseases. The Dutch TB centres focus not just on the treatment of patients with complex forms of TB and the problems associated with this, but are also active in preventing the spread of the disease, in education, in providing consultancy and advice to support other professional practitioners, and in carrying out scientific research.[9] Problems in the areas of resistance, medication, comorbidity, surgical interventions and treatment compliance are taken care of with an interdisciplinary approach. However, whether this approach leads to higher treatment success rates is not known.
Data from TB patients and treatment outcomes from Dutch TB centres have not been published previously, although in 2000 outcomes concerning multi drug resistant TB were reported from the two centres. In this article we describe the population of TB patients in Dekkerswald, admitted during the period 2000-2005, to provide insight to demographic, clinical and social characteristics.

Patients and Methods

From all patient records admitted at least two days in the period 2000-2005, demographic and clinical data were registered, as equally the reason for referral, resistance patterns and follow-up. A distinction was drawn between pulmonary TB, extrapulmonary TB, which included pleural tuberculosis, and combined pulmonary and extra pulmonary TB. The National Institute for Public Health and the Environment (RIVM) performed determination, genotyping and susceptibility tests.
'Mono-drug resistance' was defined as resistance against just one anti-TB drug and 'poly-drug resistance' as resistance against at least two drugs other than isoniazid and rifampicin. 'Multi-drug resistance' was defined as resistance towards at least isoniazid and rifampicin. Patients of foreign origin were defined as patients not born in The Netherlands.
A serious adverse effect was defined as each reaction that resulted in the suspension of the treatment with one or more anti-TB drugs or other medication, except for analgesics.

Hepatotoxicity was defined as an increase in the serum transaminase activity (aspartate aminotransferase (ASAT) or alanine aminotransferase (ALAT)) above 50 U/l and severe hepatotoxicity as transaminase values above 500 U/l. Inflammatory immune-reconstitution syndrome in HIV-positive patients was defined as a paradoxical deterioration of pre-existent infectious diseases after the start of highly active antiretroviral therapy (HAART). Comparisons between groups were assessed using the chi-squared test.

Results

A total of 166 patients were included in the study. Table 1 provides general and demographic details of the study population, classified according to land of origin. The population consisted of 37 different nationalities; 14% (n=23) were from Surinam, Turkey or Morocco. Referral. Severe hepatotoxicity was the principal reason for referral (n=18) followed by delayed response to treatment (n = 17), surgical interventions mainly for spondylodiscitis (n=12), combined HIV-TB infection (n=11), resistance problems (n=11) and need for isolation (n=9). Social reasons for referral included homelessness and substance abuse.

Table 1. Characteristics of 166 tuberculosis (TB) patients admitted to Dekkerswald, Nijmegen in the period 2000-2005.

	Origin		
	Netherlands (n=50)	**Abroad (n=116)**	**Total (%) N = 166**
Gender			
Male	35	80	115 (69)
Female	15	36	51 (31)
Mean age in years (limits)	56.3 (15-90)	34.2 (16-74)	40.8 (16-90)
Referral indication			
Clinical reason	33	80	113 (68)
Social reason	17	36	53 (32)
Referred by			
Other hospital	29	69	98 (59)
Municipal health service	11	30	41 (25)
General Practitioner	2	3	5 (3)
Radboud UMC	6	12	18 (11)
Other	1	0	1 (1)
Via outpatients clinic Dekkerswald	1	2	3 (2)
Sensitivity; no. strains	(n=49)	(n=116)	(n=165)
Normal	42	73	115 (69.7)
Mono-drug resistant*	2	14	16 (9.7)
Poly-drug resistant**	0	5	5 (3.0)
Multi-drug resistant***	0	14	14 (8.5)
Unknown	5	10	15 (9.1)
HIV	(n=49)	(n=114)	(n=163)
Positive	3	14	17 (10)
Negative	24	77	101 (62)
Not tested	22	23	45 (28)
Diagnosis			
Pulmonary TB	39	59	98 (59)
Extrapulmonary TB	5	34	39 (23)
Pulmonary and extrapulmonary TB	6	23	29 (17)

* Resistant to just one anti-TB drug
** Resistant to at least two drugs other than isoniazid and rifampicin
*** Resistant to at least isoniazid and rifampicin

Anti-tuberculosis drugs resistance
For 93% of the patients (n=155) TB diagnosis was confirmed with a positive culture result (the gold standard); for 11 patients this was not possible. In these non-culture confirmed TB cases clinical and radiological response to treatment was observed.
Resistance patterns were determined for 150 strains; in five cases the data were missing in the medical files. Thirty-five strains (23%) had an abnormal drug susceptibility. Resistance against isoniazid (n=18), rifampicin (n=15) and streptomycin (n=20) were the most frequent. Of the resistant strains, 94% (33/35) were isolated from patients of foreign origin (p<0.0001). Seventeen patients had previous TB treatment in their case history. Nine of them had an abnormal resistance pattern and two had multi-drug resistant TB. Poly-drug resistant TB occurred more frequent in patients who underwent previous treatment. Three patients who had previous treatment a reactivation could be established because DNA fingerprints concurred with those of the earlier episode. For the other 14 patients these data were missing.

HIV and TB. Equal numbers of Dutch and foreign patients were not tested for HIV. This was mainly the case in patients with a negative test result in the past or older patients with a lower prior chance of HIV infection.
Immune reconstitution syndrome only occurred in those patients who started HAART during the first 2 months of the TB treatment (8/17).

Adverse effects. Table 2 shows the adverse effects that occurred in the study population. Sixty-six (40%) of the patients were treated with second-line anti-TB drugs due to adverse effects or resistance. In 53 patients one or more of these drugs became a permanent part of the treatment.
The most prescribed were amikacin, levofloxacin and moxifloxacin. Fourteen HIV-positive patients (82%) experienced adverse effects during treatment, mainly hepatotoxicity. Almost 60% of them (n=10) had therapy changes as a result of adverse effects and interactions.

Extrapulmonary TB. Foreign born patients presented significantly more with extrapulmonary TB (p <0.01). In particular, patients from African countries (p <0.02) and Eastern Mediterranean regions (p <0.001) had more extrapulmonary TB. For many patients it concerned more than one extrapulmonary localisation, as illustrated in Table 3.
In our population, HIV-positive patients did not present significantly more extrapulmonary TB than HIV-negative patients.

Admission period. The median admission period for the total population was 10.1 weeks (limits 0.6 - 38.1). There was no significant difference in duration of admission between patients who were admitted for clinical or social reasons or between patients of Dutch or foreign origin.
HIV patients, however, were admitted longer (median 15.7 weeks; limits 10.1 - 28.0) than HIV-negative patients (median 10.6 weeks; limits 0.6-38.1 weeks). **Treatment success.** Hundred and forty one patients (85%) completed treatment (TB patients are only officially considered cured if no reactivation has occurred after 2 years). In six percent of the cases (n=10) no data concerning treatment completion after discharge from hospital were found. TB-related mortality was 5% (n=8).

Table 2. Adverse effects of anti-tuberculosis drugs in 166 tuberculosis patients admitted to Dekkerswald, Nijmegen, during the period 2000-2005

Adverse effect	**n (%)**
None	77 (46)
Unknown	3 (2)
Hepatotoxicity	64 (39)
Skin reactions	7 (4)
Neurotoxicity	5 (3)
Arthritis/gout	4 (2)
Ototoxicity	2 (1)
Eye disorders	1 (1)
Isoniazid fever	1 (1)
Allergic response	1 (1)
Drug fever	1 (1)
Total	**166 (100)**

Discussion

In this era of a gradually decreasing TB incidence, patient care appears to be increasingly concentrated in specialised centres. Dekkerswald is one of these centers and wishes to retain this expertise in the future. The patient population is characterised by complexity as a consequence of (multi)drug resistance, or HIV co-infection, adverse effects, extra pulmonary TB and social problems. Despite this complexity, the interdisciplinary approach is leading to high treatment success rates in our study population.
Virtually the entire population was referred from other hospitals and from municipal health services. The 3 patients from the population who were admitted via our own outpatients clinic are typical of the number of patients that present to the average pulmonologist in The Netherlands. [7]

Table 3. Extrapulmonary TB localisations in 68 of 166 tuberculosis patients admitted to Dekkerswald during the period 2000-2005.

Extra pulmonary localisation*	**n (%)**
Lymphnode	25 (28)
Spine	16 (18)
Pleura	11 (13)
Central nervous system	9 (10)
Miliary tuberculosis	8 (9)
Gastro-intestinal localisation	5 (6)
Musculoskeletal localisation	2 (2)
Pericardium	2 (2)
Urogenital tract	2 (2)
Ear Nose Throat area	1 (1)
Hepatobiliary area	1 (1)
Total	**88 (100)**
*More than one localisation could occur per patient	

Resistance problems are an important reason for referral to Dekkerswald. Multi drugresistant TB is a very rare phenomenon in the Netherlands (1-2% per year, which is equivalent to 10-12 cases per year).[7] In the study population, however, multi drugresistance was far more prevalent. Interestingly 85% of the multi drugresistant TB patients had good treatment outcome. This is high compared to the European and international figures[9-14] and illustrates the importance of treating multi drugresistance in specialized centres.[12,15-16]
A concomitant infection with HIV and TB is also rare in the Netherlands (n = 60; 5% of the total number of TBC patients in the Netherlands/year).[7] In the study population this percentage was ten percent. Not all patients were tested for HIV although that is recommended as standard policy. The patient group described was complex due to the large number of adverse effects, interactions and immune reconstitution syndrome. This was translated in a longer median admission period.
The low incidence of TB in The Netherlands leads to decreasing experience of treating physicians with toxicity and adverse effects of treatment. The occurrence of adverse effects therefore often results in consultation of our centre. A proportion of these patients are subsequently referred to Dekkerswald due to the complexity of their case, the expected longer admission period or the use of second-line anti-TB drugs. Hepatotoxicity is the most frequent adverse effect. The definition of hepatotoxicity of transaminase level increases > 50 U/l appears to be very strict. For an asymptomatic transaminase increase up to maximum of 5 times the reference value, no adjustment of the medication policy is, in principle, necessary.[17] Only if a rise in the transaminase level is accompanied by symptoms or is greater than 5 times the reference value, there is an indication for temporarily suspending the administration of the medication.[18] Recently, a high risk of hepatotoxicity was described for HIV-positive patients.[19] In our study population, patients with HIV co-infection also experienced relatively more hepatotoxicity.

The presence of extrapulmonary TB within the population described did not deviate from that of the Dutch population in general. In recent years this has been about 40%.[20-21] Patients of foreign origin more often have extrapulmonary TB;[20,22] this was also seen in the study population. In recent years there has been an increase in the prevalence of bone and joint TB in The Netherlands within the group of extrapulmonary TB.[23] This increase is 5-7% and is mainly visible in older (> 65 years) Dutch patients.[7] Due to a possible indication for surgical treatment in the case of spondylodiscitis[24] this was often a reason for referral to Dekkerswald. Social factors are an important reason for referral to our centre. Illegal immigrants, prisoners, alcohol and drug addicts, and the homeless often need intensive supervision to complete treatment. In these groups the chances of not completing treatment are higher with consequently an increased risk of recurrent TB or acquired resistance.[25-26]
An interdisciplinary team of nurses, a social worker, dietician, activities supervisor, pastoral care worker and a physiotherapist supervise these patients intensively. Within the study population no difference was seen in treatment result or admission period between patients with a clinical or a social reason for admission.
Treatment results, based on percentages of completed treatments, were slightly higher in our centre than the national average (86% versus 84%)(7). The mortality was the same(7). In view of the complexity and comorbidity within our population a lower success rate or a higher mortality could be expected.

In conclusion, the population of TB patients admitted to Dekkerswald had good treatment results despite the complexity and diversity of the population. In view of the number of admissions to our centre a need would appear to have been met.
The sanatoria might have disappeared from the Netherlands27,[27] but TB will never disappear completely. Especially with decreasing incidence there is an ongoing need for TB referral centres where knowledge and experience can be maintained due to the concentration of patient care, consultations, education and research. Furthermore the current international threat of extensive drugresistant TB poses a challenge to Dutch healthcare with respect to anticipation of prevention and treatment.

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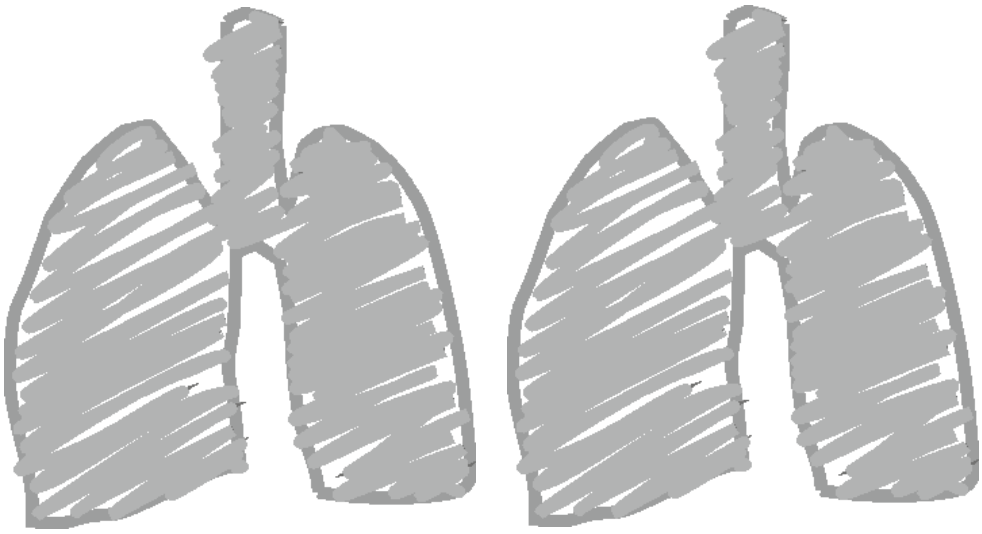
Epidemiology of *Mycobacterium bovis* disease in The Netherlands from 1993-2007

Chapter 3



Abstract

In the Netherlands, 1.4% of the tuberculosis (TB) cases is caused by *Mycobacterium bovis*. After we admitted three patients with *M. bovis* infections in our reference hospital we conducted a retrospective analysis of all *M. bovis* disease in the Netherlands during 1993-2007. We analysed data from 231 patients for clinical, demographic, treatment and outcome characteristics and for risk factors.
Most patients were native Dutch (n=138; 59.7%), or Moroccan (n=54; 23.4%). Disease was mainly extrapulmonary (n=136; 58.9%). Although 95 patients had pulmonary disease, person-to-person transmission did not occur, as shown by structural DNA fingerprinting analysis. Lymph node TB was more likely to develop in women (p< 0,0001), whereas pulmonary *M. bovis* disease developed more frequently in men (p< 0,0001). Diagnosis was accurate but delayed and led to inadequate treatment in 26% of the cases, in terms of content and duration. Proportion of deaths from *M. bovis* disease was higher than that for *M. tuberculosis* disease.

Introduction

Mycobacterium bovis disease was common in the Western world in the era before pasteurization of milk products. In 1938, the percentage of *M. bovis* disease among all patients with tuberculosis (TB) was 9% in Amsterdam and 11% in the rest of The Netherlands. In 1940, pasteurization became obligatory and in 1952, the percentage of *M. bovis* disease had dropped to 1.5-2.0% in Amsterdam.[1-2] From 1945 to the mid-1960s, *M. bovis* was gradually eradicated in livestock in The Netherlands. Nevertheless, during 1973-1975 around 2.5% of the human TB cases in The Netherlands were still caused by *M. bovis.*[3]
Since 1993, only ten cases of *M. bovis* infections have occurred in livestock in The Netherlands. All were caused by infected livestock that have been imported (D. Bakker, personal communication). Because most *M.bovis* infections are contracted through the oral route, extrapulmonary manifestations were primarily observed. Diagnosing extrapulmonary TB is generally difficult, and as the prevalence of TB has declined, the experience of physicians in diagnosing this specific infectious disease has also decreased, and therefore the time to diagnosis has increased.[4-5] The difficulties in the diagnosis and treatment of *M. bovis* infections in 3 female patients in the University Centre for Chronic Diseases Dekkerswald prompted the present study. To investigate the magnitude of *M. bovis* infections in persons in The Netherlands, we conducted a retrospective study of patients with *M. bovis* infections and describe the epidemiologic, clinical and bacteriologic findings.

Materials and Methods

We retrieved data from two databases; one at the National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, which contains the bacteriologic information of all *M. tuberculosis* complex isolates in the Netherlands. The other is the Netherlands Tuberculosis Register (NTR), an anonymous case register held by KNCV Tuberculosis Foundation in The Hague, The Netherlands.
This database is based on a voluntary registration, but contains data of virtually all TB patients in The Netherlands. The patients in the NTR database have been registered by their physicians. This register holds basic information on demographic, clinical and some bacteriologic data. Death is registered as TB-related or not-TB-related without further explanation. Treatment and treatment outcome are registered without further details. Exact treatment length could be determined because length was registered in 2 different categories. Therefore, we could categorize treatment length in 6 groups, namely <3 months, 4 -5 months, 6 months, 7-9 months, 10-12 months and > 12 months.
We selected all *M. bovis* cases that occurred during 1993 - 2007. By using these two databases we had information on bacteriologic and clinical factors, demographic data, risk factors, as well as treatment and outcome. We used the RIVM database for the bacteriologic data and localization of the infections and the NTR database for the demographic and clinical data, including treatment outcome.
To distinguish *M. bovis caprae* and *M. bovis bovis* cases, we reviewed available IS*6110* restriction fragment length polymorphism (RFLP), spoligotyping and pyrazinamide susceptibility data of all *M. bovis* isolates analysed in this study.[6-7]

Because IS*6110* RFLP typing of all *M. tuberculosis* complex isolates is routinely performed in the Netherlands, we also analyzed information on possible interhuman transmission of *M. bovis*. Associations between localisation of infection in the patient, geographic distribution, ethnicity, etc. were evaluated on basis of information from both databases by using the X^2 test. Mortality rates were evaluated by using the NTR database and were correlated with the demographic and clinical data.

Case reports

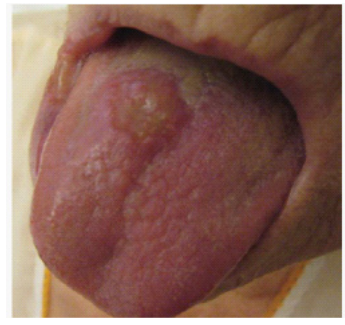
Patient 1

A 73 year old female patient sought treatment for non-productive cough and dyspnoea. Medical history included severe rheumatoid arthritis, for which she had received treatment with anti-Tumor Necrosis Factor-α (anti-TNF-α) treatment for 1 year. High resolution Computed Tomography (CT) imaging of the thorax showed micronodular lesions in both lungs. CT imaging of the brain indicated several cerebral nodular lesions, suspected to be tuberculomas. Culture of broncho alveolar lavage fluid yielded *M. bovis*. The patient was treated with isoniazid (H), rifampin (R), ethambutol (E). Moxifloxacin and steroids were added, because of the high penetration level through the blood-brain barrier and to compensate for the absence of pyrazinamide in the treatment regimen.

Patient 2

An 84 year old female patient with a medical history of myelodysplastic syndrome and diabetes mellitus type 2 sought treatment for a traumatic leg wound that did not heal, and her condition gradually progressed to generalized skin lesions. She was referred to the dermatologist in our hospital. Culture of a skin biopsy grew *M. bovis.* The patient had lesions on her tongue, typical for TB (see Figure 1), and in her larynx and lungs. The patient's condition resolved after 9 months of treatment with isoniazid, rifampicin and ethambutol. Contact tracing showed no human transmission.

Figure 1. Mycobacterium *bovis* lesion of the tongue of patient 2.



Patient 3
An 87 year old female patient with severe rheumatoid arthritis sought treatment for weight loss, cough, night sweats and dyspnoea one year after starting anti-TNF-α treatment. Radiologic imaging showed a single nodular lesion of the lung parenchyma and pleural fluid. Culture of a broncho alveolar lavage specimen yielded *M. bovis*. The patient received isoniazid, ethambutol and moxifloxacin for 18 months because she had an intolerance for rifampicin. No contact tracing was performed.

Results

During 1993-2007, a total of 16,059 patients were registered in the NTR with culture-proven tuberculosis; 231 (1.4%) patients were registered with *M. bovis* infection[8] (Figure 2).The number of patients with *M.bovis* infection born outside The Netherlands (foreign born) showed an increase in the proportion from 35.1% to 46.3% in the last five years of the study period (Figure 3).
Over time, the percentage of patients with *M. bovis* TB who live in major cities increased from 33.3% to 41.8%, with an accompanying decline elsewhere in The Netherlands. No relation was found between outbreaks in livestock and human disease.

Figure 2. *Mycobacterium tuberculosis* and *M. bovis* infections, The Netherlands, 1993-2007. Data derived from the National Institute for Public Health and the Environment (RIVM) database.

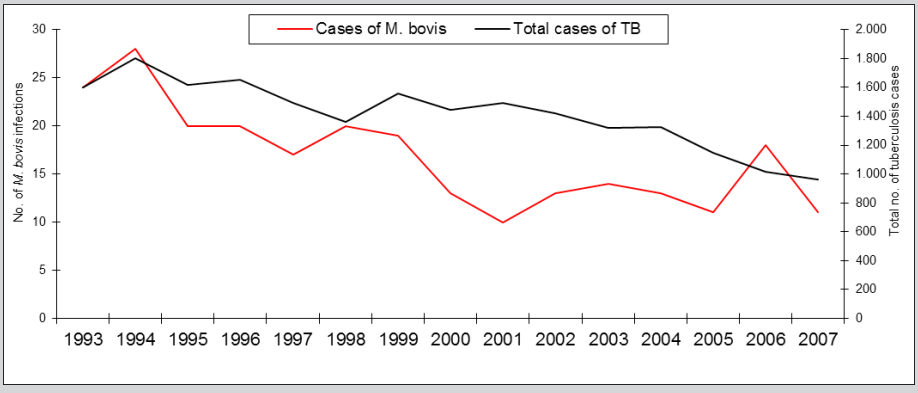
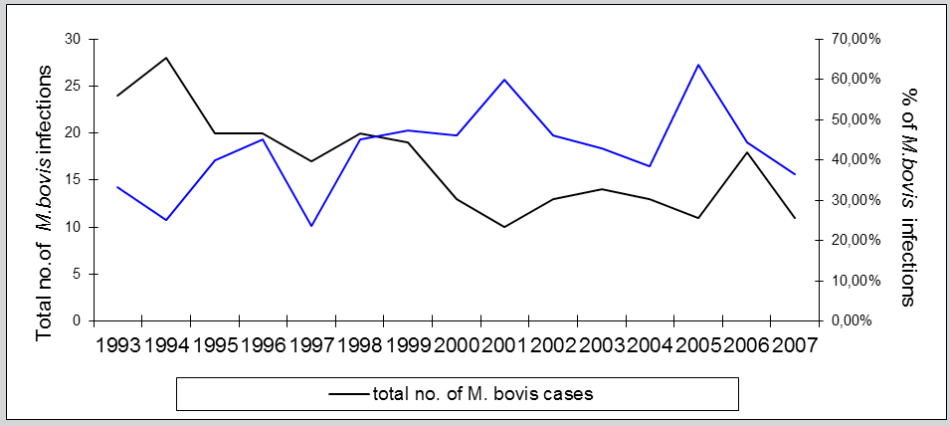
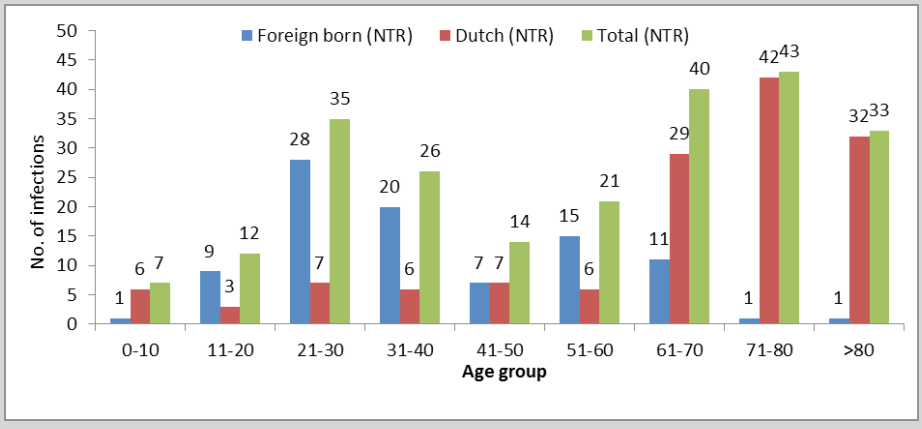


Figure 3. Percentage of *Mycobacterium bovis* infections in foreign born persons and total number of *M. bovis* infections, The Netherlands, 1993-2007. Data derived from the National Institute for Public Health and the Environment (RIVM) database.



Among patients with *M. bovis* disease, a significant difference in mean age was noted among those born in The Netherlands and immigrants. In native Dutch persons with *M. bovis* disease, the median age was 64.8 years whereas among immigrants with *M. bovis* disease the median age was 38.6 years (Figure 4).
We observed 58 cases in young foreign-born patients and 16 cases in young Dutch patients (< 40 years). Five Dutch patients had a general risk factor for contracting *M. tuberculosis* complex disease (one drug addiction, one alcohol addiction and three not specified risk factors) and 44 foreign-born had 1 or 2 general risk factors (32 immigrants, 8 fugitive status, 3 illegal immigrants, and 1 TB-contact, 1 in criminal detention, 1 not specified risk factor).

Figure 4. Number of Mycobacterium bovis infections, according to patient age and origin, The Netherlands, 1993-2007. Data derived from the NTR database.



Most of the patients were native Dutch (138 out of 231; 59.7%). Most foreign-born patients came from Morocco (54 out of 93; 58.0%). The other immigrants had other places of origin: Europe (n= 6; 6.5%), Africa (n= 15; 16.1%), Asia (n= 9; 9.7%), and South America (n= 3; 3.2%) (Table 1). A distinction between native Dutch and second generation Dutch immigrants could not be made.

Bovine tuberculosis mainly had appeared as extrapulmonary disease; 136 (58.9%) of 231 had extrapulmonary disease alone, 68 (29.4%) of 231 had pulmonary *M. bovis* disease and 27 (11.7%) of 231 had both pulmonary and extrapulmonary disease. A significant difference between men and women was found in terms of localization of disease. In women lymph node TB was more likely to develop, mainly located in the cervical region (p<0,0001); men more often had pulmonary *M. bovis* disease(p<0,0001). This finding was seen in all age groups in both Dutch and foreign-born patients (Table 1).

Table 1. Clinical and demographic data derived from the NTR database

Bovine tuberculosis	**Netherlands**		**Foreign-born**		**Total**
Sex					
Male	56 (40.6%)		49 (52.7%)		105 (45.5%)
Female	82 (59.4%)		44 (47.3%)		126 (54.5%)
	Male	**Female**	**Male**	**Female**	
Age					
0-60	13	22	45	35	115 (49.7%)
60+	43	60	4	9	116 (50.3%)
Localisation					
Pulmonary tuberculosis	26	22†	25	7‡	80 (34.6%)
Respiratory tract	5	8	5	1	19 (8.2%)
Menigeal and CNS	2	4	0	0	6 (2.5%)
Intestinal tract	0	5	3	5	13 (5.6%)
Bone and joint TB	4	5	2	1	12 (5.2%)
Urogenital tract	4	4	3	2	13 (5.6%)
Other organs (f.e.: lymph node)	5	24*	9	21∫	59 (25.5%)
Miliary TB	6	3	0	2	11 (4.8%)
Unknown	4	7	2	5	18 (7.8%)

Foreign-born patients originate from European countries (n= 6), Africa (n= 69), Asia (n= 9), South America (n= 3), and Unknown (n= 6)

† p=0.018

‡ p<0.001

* p=0.004

∫ p= 0.002

Several treatment schedules were used for our study population; 157 of 231 patients received a combination of isoniazid (H), rifampin (R), ethambutol (E) and pyrazinamide (Z) or another pyrazinamide containing antibiotic combination. Treatment with pyrazinamide-containing regimens had no negative effect on treatment outcome.
According to World Health Organization (WHO) standards, a regimen of isoniazid and rifampicin of 9 months and ethambutol for 2 months is indicated for treating *M. bovis* disease. In our study population, 113 (52%) of 217 patients were cured and completed treatment according to the WHO standard. Thirty seven (17%) of 217 patients were considered cured with an insufficient treatment schedule, duration or both.
For 14 patients, outcome data were not available. No significant associations were found no significant associations between insufficient treatment and particular disease localizations or outcome. An anonymous questionnaire distributed to the physicians who were caring for the patients at the time of the disease (34 sent and 24 returned) showed that most of the physicians followed the Dutch TB guidelines, which are based on the WHO standard. A problem these colleagues faced was the lag of time between sampling and the results of culture, identification and drug susceptibility test results, which sometimes took as long as 4 months. Treatment outcomes comparisons for Dutch and foreign-born patients in The Netherlands are shown in table 2.
The overall proportion of deaths for the infection itself was 5.2% for *M.bovis* disease and the proportion of deaths associated with non-TB-related causes (e.g. cardiac disease and haematological malignancy) for patients with *M. bovis* was 14.7%.

Table 2. Treatment result according to age, sex and localisation of disease in Dutch and foreign-born patients as recorded in the NTR database.

Bovine tuberculose	**Netherlands**				**Foreign-born**			
Tretment result	**Treatment completed**	**Cause of death**		**Other**	**Treatment completed**	**Cause of death**		**Other**
		TB related	**NonTB related**			**TB related**	**NonTB related**	
Total n (%)	85 (61.6)	10 (7.2)	28 (20.3)	15 (10.9)	66 (71.0)	2 (2.1)	6 (6.5)	19 (20.4)
Age								
0-60	25	1	3	6	59	2	3	16
60+	60	9	25	9	7	0	3	3
Sex								
Male	33	3	18	2	35	0	3	11
Female	52	7	10	13	31	2	3	8
Localisation								
Pulmonary	26	2	9	3	21	0	2	5
Extrapulmonary	52	5	11	10	44	0	3	11
Pulmonary and Extrapulmonary	7	3	8	2	1	2	1	3

Other: treatment not completed and treatment continued elsewhere

An analysis of the overall mortality from bovine TB according to sex, age, ethnicity and disease localization is shown in table 3. A correlation was found between death and age > 60 years, Dutch nationality, and miliary disease . When dividing death in the categories of TB-related and non-TB-related, only high age was statistically significant between the groups; all other variables were not significant (Table 4). Cause and rate of death differed between sexes; among women, death was more often related to *M. bovis* TB; in men, the overall mortality rate was higher, although these differences were not statistically significant.

Table 3. Correlation of overall mortality with demographic variables*

Mortality	p-value
Sex	0.31
Age >60 years	< 0.0001
Dutch nationality	0.001
Disease localisation (miliairy TB)	< 0.0001

* X^2-test

Table 4. Correlation of TB related and non-TB related mortality with demographic variables*

Mortality	p-value
Sex	0.58
Age >60 years	0.03
Dutch nationality	0.91
Disease localisation (miliairy TB)	0.49

*X^2-test

Discussion

Bovine tuberculosis comprised 1.4% of all TB cases in The Netherlands during 1993-2007 This finding is comparable to those of studies in other countries where control and corresponding control efforts of *M. bovis* TB in livestock are present.[9-11]
During 1993-1997, mainly elderly Dutch persons were found to have bovine TB; later (1998-2007) the infections were divided more equally between the native Dutch and immigrants (Figure 3). *M. bovis* TB in the population of The Netherlands shows an age-curve with double peaks. The younger patients were mostly foreign-born or first- and second-generation immigrants, who may have (frequently) travelled back to their country of origin or contracted an oral *M. bovis* infection before coming to the Netherlands.

These patients more often had extrapulmonary *M. bovis* disease mainly affected the cervical or abdominal lymph nodes. Ingestion of non-pasteurised dairy products is the most likely route of infection.[12-13] The 12 Dutch patients, ages 20 - 40 years, may have been in contact with non-pasteurised food while travelling to developing countries, because the chance of contracting a *M. bovis* infection in The Netherlands is considered low.
The second age-peak contains elderly native-born Dutch persons. This result is most likely a birth-cohort effect because these persons most probably had a late endogenous reactivation of latent *M. bovis* infection contracted in the era before pasteurization of milk was introduced and while *M. bovis* infection in livestock was still highly prevalent in The Netherlands. Spoligotyping of their isolates showed a single typical pattern, which is considered the old predominant cattle-endemic strain of the Netherlands.[14] This epidemiological trend is also seen in other European countries including Norway, Sweden and Belgium.[9-10]
Endogenous reactivation of *M. bovis* infections in elderly patients follows impairment of immunity from hematological causes, immune modulation by medication including anti-TNF-α treatment, other comorbidity or immunosenescence. As the use of anti-TNF-α treatment rises, due to increasing indications in rheumatologic and gastrointestinal diseases, mycobacterial disease will likely become more common.[15] Two of the patients we described had negative tuberculin skin test (for *M. tuberculosis complex*) and chest radiograph results, and therefore did not receive isoniazid prophylaxis before the received anti-TNF-α treatment.
This result calls into question the efficacy of the screening protocol used in the Netherlands to evaluate patients before they receive anti-TNF-α treatment. Of note, reactivations of latent TB usually occur in the first 3-4 months of anti-TNF therapy,[15] although in our 2 *M. bovis* patients, reactivation occurred after 1 year. This long-term reactivation of diseases has been previously observed[16-17] and raises questions involving the role of the bacteriologic virulence factor in the pace of the reactivations.

In the Netherlands, the largest 2 immigrant populations are from Turkey and Morocco (378,330 and 341,528 respectively).[18] A high occurrence of *M. bovis* disease was observed in Moroccan patients, whereas, to our surprise, *M. bovis* disease was rare in Turkish patients. Both populations come from agricultural areas where pasteurization of dairy products is not common. Popular raw milk cheeses in Morocco ('jben') and Turkey (kasar, tulum) have been found to contain *Listeria* and/or *Brucella* species, in as many as 8,2% of the samples tested.[19-20] In both studies cheeses were not tested for *M. bovis*.
However, *M. bovis* can survive in raw milk cheese and cause an infection after it is eaten, as was described recently in a cluster of infections resulting from consumption of fresh Mexican cheese in New York and San Diego.[12-13,21] Besides consumption of fresh cheese and unpasteurized milk, consumption of raw or undercooked meat is also a possible route of oral transmission.[10,22-23]

More women than men were infected by *M. bovis*, contrary to the epidemiology of *M. tuberculosis*. Other studies about *M. bovis* do not show this result.[9,11]
This difference could be an age-related effect for Dutch patients, however, one can also see this difference in the Moroccan patient population (data not shown). Notably, we also found a difference in disease localization between men and women.

More cervical lymph node TB, which was not correlated with age, was diagnosed for female patients. Female sex has in general been considered a risk factor for *M. tuberculosis* extrapulmonary TB.[24-26] However, this finding has not been described for *M. bovis* TB. Various explanations have been given for this gender difference in TB. One possible explanation could be a difference in the immunity to *M. tuberculosis* complex infection. Several studies have been conducted in humans to compare the immune response in both sexes. Differences in reaction among others in TNF and interleukin-10 production have been found.[27-28] Another explanation for this finding may be related to the route of infection. Men could be more likely to become infected through the tracheal route by aerosols from diseased cattle, whereas women become infected by ingesting *M. bovis* while preparing or consuming contaminated food. Lastly, smoking has also been identified as a risk factor for pulmonary TB,[25] which (assuming a higher percentage of smokers are men) could explain the difference we found in our study that men have more pulmonary *M. bovis* disease than women. Unfortunately, we could not retrieve smoking status from the databases.

The geographic distribution of bovine tuberculosis in The Netherlands showed a proportional increase in *M. bovis* infections in the major cities in the Western part of the Netherlands during 1993-2007. This finding probably reflects the population demographics of The Netherlands where most persons live in and between the major cities, but also by the fact that immigrants mainly live in the major cities and that the elderly persons who contracted bovine TB in the pre-pasteurisation era are undergoing a natural decline in health. We showed that, in 37 (17.1%) of 217 patients, treatment of *M. bovis* disease was not compliant with international guidelines. Although all *M. tuberculosis complex* isolates in The Netherlands undergo drug susceptibility testing, apparently the results do not reach clinicians. Recognition of *M. bovis* as the causative agent of TB does not in itself lead to the conclusion that adjustment of the therapy is required; many physicians only make adjustments after receiving the results of drug susceptibility testing, which usually arrive quite late because of the slow growth of *M. bovis*. Moreover, the quick molecular identification of cultured *M. bovis* still implies a culture delay of about 4 weeks. Better education of physicians and increasing future application of molecular identification of *M. bovis* directly in clinical samples, i.e. sputum or lymph node aspirates, is therefore needed. The overall mortality among patients with *M. bovis* disease was higher (19.9%) than the overall mortality among patients with *M. tuberculosis* disease (4.4%) in The Netherlands. The mortality rates during 1993-2007 were 5.2% for *M. bovis* disease versus 1.9% for *M. tuberculosis* disease. This result is probably related to the higher prevalence of miliary and central nervous system localization in *M. bovis* disease. Death rates from other, non-TB-related, causes for patients with *M. bovis* were 14.7% compared to 2.5% for *M. tuberculosis*[8] infection. Subgroup analysis of *M. bovis* showed that the proportion of deaths was higher among Dutch than in foreign-born patients, but this result most likely correlates with age and impairment of immunity.[10-11,29] We cannot explain the unexpected trend in lower survival of female patients after an episode of *M. bovis* disease. Previous studies have shown that male sex is a risk factor for unsuccessful treatment,[30-31] but the numbers in our study were too small to draw any firm conclusions.
This study had limitations; the analysis of the results was hampered by the relatively low number of foreign born patients (n=93) and the heterogeneous nature of the study population.

Therefore, only data concerning patients from Morocco (n=54) could be analysed separately, and differences with the Dutch case-patients were found in terms of age, mortality rate and primary localization of disease.
Another limitation is that a distinction could not be made between native Dutch and second- generation immigrants born in The Netherlands. This lack of distinction implies that some patients have roots in a foreign country where the risk for *M. bovis* TB could be higher.
In conclusion, the incidence of *M. bovis* in the Netherlands is comparable to that in other countries in which control programs for *M. bovis* are enforced. Gender differences in clinical presentation and mortality rates were found in our cohort of patients. The disease now mainly infects immigrants from Morocco and elderly Dutch citizens. Anti-TNF-α treatment is an emerging cause of endogenous reactivation of *M. bovis* disease in elderly Dutch patients, as occurred in 2 of the recent bovine TB cases described in this article; reactivation may be slower than for *M. tuberculosis*.

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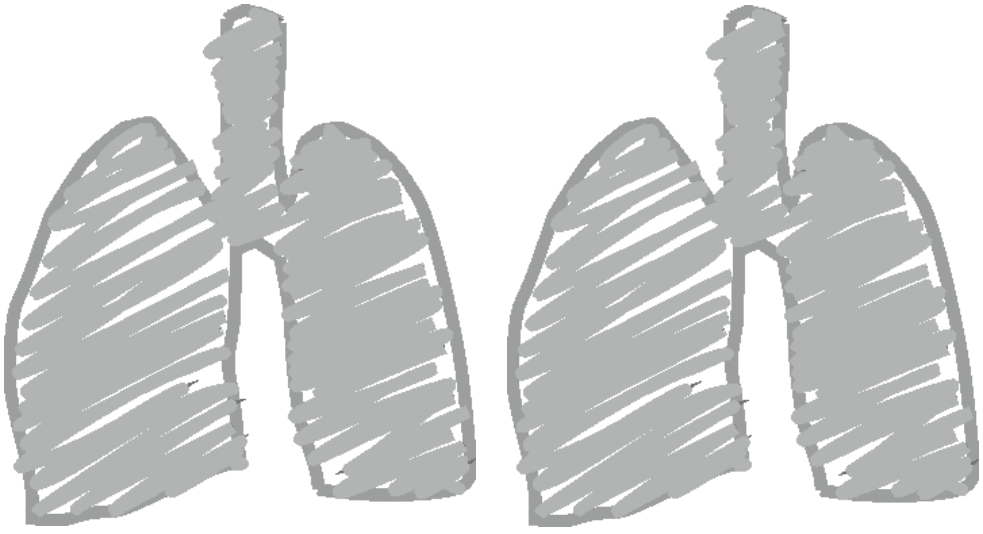
Characteristics and accuracy of non-culture confirmed tuberculosis diagnosis in The Netherlands from 2007-2009

Chapter 4



Abstract

Introduction. From 2007-2009, the proportion of culture confirmed tuberculosis (TB) cases among all notified TB cases decreased in The Netherlands from 72 to 66 percent.

Objective.We assessed the accuracy of registration of culture results in the TB register and studied the characteristics of patients notified with non-culture confirmed TB and the quality of TB diagnosis.

Methods. Patient records of all 950 patients notified with non-culture confirmed TB in the period 2007-2009 were reviewed at the Public Municipal Health Services (PMHS), whereafter hospitals and laboratories were contacted to obtain missing information on diagnostic tests. These data were compared to the information in the Netherlands Tuberculosis Registry (NTR). Cases were classified according to the TB case definition of the European Centre for Disease prevention and Control (ECDC) as; '*no', 'possible', 'probable'* or *'confirmed'* TB.

Results. Laboratory confirmation data of (191/3171=) 6% of notified TB cases in the NTR were incorrect or incomplete. The overall percentage of culture confirmed cases was corrected from 70% to 71%. In total 63% of non-culture confirmed cases were extrapulmonary TB. A total of 42% of non-culture confirmed TB diagnoses were established with other laboratory evidence such as positive Polymerase Chain Reaction or positive Ziehl Neelsen staining, or histology suggestive of TB. In total 164 out of 516 (32%) of the non-culture confirmed cases represented early diagnosis of extra pulmonary TB due to active case finding by physicians of PMHS, and no culture was attempted. In clinical extra pulmonary cases culture was not done because biopsy material was not suitable for culture anymore. After correction, according to the ECDC case definition classification, 56% of non-culture confirmed cases was classified as '*possible TB*', 35% as '*probable TB*', and 6.8% as '*confirmed TB*'. Thirteen cases could not be classified due to missing data.

Conclusion. The overall culture confirmation rate changed from 70 to 71% after correction of incorrect or missing data. TB patients with non-culture confirmed disease were more often those with paucibacillary disease and children. Most 'possible' TB cases were those with a high likelihood of infection, diagnosed through active case finding by physicians of the PMHS. 'Probable' TB cases were mostly diagnosed in the hospital, culture was attempted in 60% of the cases but remained negative. In 31% of the cases the body material was retrieved, but delivered in the wrong condition to be cultured.

Introduction

Culture of *Mycobacterium tuberculosis* is considered the gold standard in the diagnosis of tuberculosis (TB), confirming a presumptive diagnosis on the basis of a variety of clinical symptoms.[1] In the era of emerging drug resistance, culture is increasingly important to facilitate drug susceptibility testing (DST) in order to verify and adjust treatment. In The Netherlands, country wide culture and DST in the last decades have contributed to low relapse rates.[2]
In case of pulmonary TB, especially if characterized by large cavities, the diagnosis is generally robust and confirmed by culture.[3]
In a proportion of TB diagnoses, especially in (children with) extra pulmonary TB (ETB) and in HIV co-infected patients, culture confirmation is lacking.[4-5] TB diagnosis is often more challenging in those cases, due to poor accessibility of the TB lesions and a low bacterial load.[3,5-8] The overall impression of clinical symptoms, Ziehl Neelsen (ZN) or Auramine fluorescence microscopy, polymerase chain reaction (PCR) targeting *M. tuberculosis complex,* X-ray and other radiological imaging methods, histological examination, results of tuberculin skin testing (TST), interferon gamma release assay (IGRA), and ultimately response to TB treatment all provide some degree of confirmation of the diagnosis, but are not always conclusive.[3,9]
In The Netherlands, the number of TB cases is declining, as there were 1612 notified cases in 1995, 1443 in 2000 and 1007 in 2011 with a notification rate in 2011 of 6 per 100 000 population. At the same time the proportion of ETB cases is increasing, as 56% of the cases are exclusively PTB, and 44% involve ETB.[10]
Physicians of the Public Municipal Health Services (PMHS) diagnose approximately 20% of TB cases through active case finding. Other patients are diagnosed by hospital affiliated physicians, i.e. pulmonologists, internal medicine specialists and pediatricians. TB is a notifiable disease to be reported to the PMHS by the diagnosing physician and the medical microbiologist. The PMHS subsequently submit data to the central web based NTR. A TB case in the NTR is a patient diagnosed with TB after culture confirmation or with a suspicion of active TB with an indication for treatment. Surveillance data and in particular the proportion of culture confirmed cases are based on this register.
From 2007-2009, the proportion of culture confirmed TB cases among all notified TB cases decreased in The Netherlands from 72 to 66 percent, while in the period 2000-2006 72-76% of all TB cases were culture confirmed.[10] Especially in ETB, culture confirmation decreased significantly in the last ten years; from 70 to 54%. In 2008 an international review committee assessed the quality of the TB control program in the Netherlands. The committee specifically expressed a concern about the comparatively low and declining rate of culture confirmation of notified TB cases. The team recommended to study whether these findings could be related to a decreasing quality in diagnosis linked to loss of expertise in a country with a declining TB epidemic.

The objective of this study was to determine the characteristics of non-culture confirmed TB cases, to assess the accuracy of registration of culture results and TB diagnosis in the NTR and to assess the quality of diagnosis in non-culture confirmed TB cases.

Methods

A retrospective, descriptive study was performed of the notified non-culture confirmed TB cases in the NTR in the period 2007-2009. We classified the cases into 3 groupings; 1: no culture performed, 2: culture result negative, and 3: unknown whether culture had been performed or results remained unknown. Medical records at the PMHS of all cases in groups 1 and 3 were reviewed and a sample of 15% of the culture negative cases. The selection of the latter group concerned adults from four different PMHS with the largest group of patients. When indicated, physicians in the hospital or microbiological laboratories were contacted in order to obtain missing information. In case of failure to obtain sufficient information, this was registered as 'missing data'. Basic demographic data, and more detailed information on who diagnosed the TB case, symptoms, presentation of TB, co-morbidity, methods applied to diagnose TB, reasons for negative culture results or the lack of culture result in the NTR were all recorded.

We re-classified the cases based on the additional information retrieved from the patients files; group A: not culture performed, B: culture negative, C: culture positive, and D: unknown whether culture had been performed or unknown result, to enable comparison with the original NTR data.

Subsequently, based on the final laboratory information cases were classified according to the TB case definition of the European Centre for Disease prevention and Control (ECDC).[11] '*Confirmed TB*' was defined as cases with positive *M. tuberculosis* cultures or having both positive ZN as well as positive PCR results. '*Probable TB*' were those who had, in addition to signs, symptoms and/ or radiological findings consistent with active TB, a positive ZN staining or detection of *M. tuberculosis* complex in DNA or RNA amplification or granulomas in histology. '*Possible TB*' was defined as signs, symptoms and/or radiological findings consistent with active TB in any site and a clinician's decision to treat the person with a full course of anti-tuberculosis therapy. '*No TB*' was defined as registered incorrectly in the NTR. All statistical analysis was performed in SPSS version 18.0.

Results

Characteristics of total population registered in the NTR during the period 2007-2009: Of 3171 notified TB patients 70% had culture confirmed (n=2221) TB and 33.7% had ETB. Individuals of Somalian nationality, by far the largest group of immigrants with TB in recent years, showed a deviating ratio of 54.2% ETB compared to the whole population and had a slightly lower overall percentage of culture confirmed TB diagnoses (67.9%).

Children aged 0-14 years accounted for 5% (n=158) of the total population. Twenty two percent (36/158) had culture proven TB and 63% (99/158) were diagnosed during contact tracing. Less than 4% of the notified cases were HIV infected (n=114), 24% were HIV negative (n=755) and the rest had an unknown HIV status. Eighty percent had culture proven TB and 10% was culture negative.

Characteristics of notified cases with non-culture confirmed TB: Of non-culture confirmed TB cases (n = 950) 57.1% had ETB, 294 were classified in group 1, 477 in group 2 and 179 in group 3. In total 516 files were studied (285 group 1, 65 /477 (14%) group 2, 166 group 3). Twenty-two cases were excluded due to wrong diagnosis (n = 12), latent TB infections (n = 7), double registry (n = 1) and missing files (n = 2).

Table 1 shows the characteristics of the non-culture confirmed TB cases after studying and re-classifying the patient records at PMHS. In group 3 the highest incidence of erroneous registries was found. After studying the patient records, only 16% of the patients remained with unknown results. Of these cases 25 out of 29 (86%) were diagnosed by a pulmonologist and only one (3.4%) by a physician of the municipal TB control service.

In 17.8% of cases children aged 0-14 years were involved. PTB accounted for 37.4% and ETB for 62.6% of cases. Thirty cases were treated for TB in the past, the majority (16; 53.3%) was cultured but resulted negative, 11 (36.7%) were not cultured. In nine of these formerly treated cases, no material was obtained in the procedure at the PMHS and in two cases material was delivered in the laboratory in wrong conditions. TB physicians from the PMHS diagnosed 139 patients (48%) of the cases in group 1. Forty-one percent of these diagnoses (119/290) were "early diagnoses" from screening risk groups and contact investigations, of which 66 were children.

Next to the physicians at the PMHS, pulmonologists and specialists in internal medicine accounted for respectively 113 (39%) and 25 (9%) of the diagnoses established without culture results (group A). In 44 out of 113 cases (38.9%) the pulmonologist did not perform *Mycobacterium* cultures (of which in total 32 cases of uveitis), in five patients material was not obtained, and from ten patients the sample never arrived in the laboratory. As a final group of physicians, pediatricians diagnosed 7 out of 84 children with TB in group A. In five of these cases they did not perform cultures.

Positive histology for TB was indicated in 147 (28.5%) and positive PCR in 53 (10.2%) of non-culture confirmed TB cases in the study group. From all patients with incorrectly delivered materials (n=71), 56 showed granulomas with 21 cases with positive PCR, 9 negative PCR and 26 not performed. No patients had negative histology and positive PCR. A total of 42% of diagnoses were established with other supportive evidence than culture (data not shown in table 1).

Table 1. Main characteristics of cases after re-classification into groups A-D

Patient characteristics*		Culture result				Total
		Group A 'no culture performed' (n=290)	**Group B** 'culture negative' (n=184)	**Group C** 'culture positive' (n=13)	**Group D** 'unknown result' (n=29)	(n=516)
Age groups	0-14	84 (91.3)	7 (7.6)	0 (0)	1 (1.1)	92 (100)
	15-44	121 (49.6)	102 (41.8)	6 (2.5)	15 (6.1)	244 (100)
	45-64	50 (47.2)	44 (41.5)	6 (5.7)	6 (5.7)	106 (100)
	>65	35 (47.3)	31 (41.9)	1 (1.4)	7 (9.5)	74 (100)
Co-mmorbidity	HIV positive	5 (41.7)	6 (50)	1(8.3)	0 (0)	12(100)
	Other¶	29 (38.7)	35 (46.7)	5 (6.7)	6 (8.0)	75(100)
type of TB	PTB	68 (48.2)	65 (46.1)	4 (2.8)	4 (2.8)	141(100)
	ETB	209 (64.7)	89 (27.6)	5 (1.5)	20 (6.2)	323 (100)
	P + E	13 (25)	30 (57.7)	4 (7.6)	5 (9.6)	52(100)
TB status	New	255 (59.6)	149 (34.8)	7 (1.6)	17 (4.0)	428 (100)
	Previously treated	11 (36.7)	16 (53.3)	1 (3.3)	2 (6.7)	30 (100)
	Unknown	24 (41.4)	19 (32.8)	5 (8.6)	10 (17.2)	58 (100)
reason of diagnosis	Active case finding	119 (72.6)	43 (26.2)	2 (1.2)	0 (0)	164 (100)
	Passive case finding	153 (47.5)	130 (40.4)	11 (3.4)	28 (8.7)	322 (100)
	Unknown	18 (60.0)	11 (36.7)	0 (0)	1 (3.3)	30 (100)
Diagnosing physician	PMHS	139 (75.5)	43 (23.4)	1 (0.5)	1 (0.5)	184 (100)
	Pulmonologist	113 (44.1)	112 (43.8)	9 (3.5)	22 (8.6)	256 (100)
	Other clinicians	37 (51.4)	29 (40.3)	3 (4.2)	3 (4.2)	72 (100)
	Missing	1 (25)	0	0	3 (75)	4 (100)
Histology	Characteristic TB	72 (49.0)	63 (42.9)	3 (2.0)	9 (6.1)	147 (100)
	Negative for TB	2 (22.2)	6 (66.7)	0 (0)	1 (11.1)	9 (100)
	Not done/ result unknown	216 (60.3)	115 (32.1)	10 (2.8)	17 (4.7)	358 (100)
PCR	Positive	28 (53.8)	18 (34.6)	5 (9.6)	1 (1.9)	52 (100)
	Negative	15 (31.3)	31 (64.6)	0 (0)	2 (4.2)	48 (100)
	Not done/ result unknown	247 (63.7)	134 (34.5)	2 (0.5)	5 (1.3)	388 (100)
Culture	Not sampled	184				184
	No material obtained	17				17
	Material not to lab	12				12
	Wrong condition	71				71
	Missing	6				6

**absolute numbers and row percentages*

¶ other co-morbidities: diabetes mellitus, malignancy, renal insufficiency and immune suppressive drugs.

Validity of the Netherlands tuberculosis registry: In table 2 the accuracy of registry in the NTR is showed. Of the cases in group 1, 2 and 3 respectively 87%, 91% and 10% were registered correct in the NTR. Overall, only 5.6% (29/516) of the cases remained with unknown culture results or diagnostics after the study. Of culture positive cases 12/13 (92%) were diagnosed in the hospital. Almost all of these results could be easily extracted from the PMHS patient record. The exact reason why they did not feature in the NTR did not become clear in our study. These 13 culture positive cases modify the overall percentage of culture confirmation in the NTR from 70.0 to 70.9%. Laboratory confirmation data of 6% (191/3171) of notified TB cases in the NTR were incorrect or incomplete. As 91% of the random sample of the culture negative group were registered adequately, a total of 60% ((477:100X91) + 30 + 95 = 559/928) of the non-culture confirmed TB cases may be assumed truly culture negative during 2007-2009. The reasons why cultures were negative could not be extracted from the patient files and it was not possible to obtain this information from the clinicians or medical microbiologists.
From the culture negative cases 'group 2'; 477 minus 65 (random sample) cases were not studied. As a result we did not validate 13% of all cases from 2007-2009.

Table 2. *Accuracy of national tuberculosis registry of non-culture confirmed TB cases*

NTR data	After re-classification: group A-D
Group 1 n= 285	Group A 'no culture performed' n = 248 (87%)
	Group B 'culture negative' n = 30 (11%)
	Group C 'culture positive' n = 1 (0.3%)
	Group D 'unknown result/unknown whether culture performed' n = 6 (2.1%)
Group 2 n=65/ 477	Group A 'no culture performed' n = 1 (1.5%)
	Group B 'culture negative' n = 59 (91%)
	Group C 'culture positive' n = 0 (0%)
	Group D 'unknown result/unknown whether culture performed' n = 5 (7.7%)
Group 3 n= 179	Group A 'no culture performed' n = 41 (22.9%)
	Group B 'culture negative' n = 95 (53.1%)
	Group C 'culture positive' n = 12 (6.7)
	Group D 'unknown result/unknown whether culture performed' n = 18 (10.1%)

Group 1 *no culture performed,* **group 2** *culture negative result,* **group 3** *unknown result or unknown if culture was performed.*

NTR *Netherlands tuberculosis registry,* ***PMHS*** *public municipal health service*

Group A *no culture performed,* ***group B*** *culture negative,* ***group C*** *culture positive,* ***group D*** *unknown result*

Quality of TB diagnosis: According to the ECDC case definition, 55.8% (n=288/516) was classified as '*possible TB'*, 34.8% (n=180) as '*probable TB'*, and 6.8% (n=35) as '*confirmed TB'*; 13 cases were culture confirmed and 22 cases were confirmed as a consequence of the more extensive definition of the ECDC comprising positive ZN and PCR as a regular method to diagnose TB. The reason why culture remained negative in these 22 cases did not become clear from our study.
Thirteen cases could not be classified due to missing data. In Table 3 the characteristics of the patients in the study population with '*possible*' and '*probable*' TB diagnoses are outlined. From the '*possible TB'* cases, 139 out of 288 (48%) cases were identified through screening of risk groups and contact tracing. Hundred and sixty-nine of the 288 (68%) of the cases were diagnosed and treated by a physician of the PMHS.
In the group classified as '*probable TB'*, 63% had ETB, 11% pulmonary and extrapulmonary TB localizations (P+E) and 26% had PTB. Culture was negative in 109 out of 180 patients (61%). Subsequently, the diagnosis was based on positive results for ZN microscopy of other material than sputum (20%), PCR (29%) and/or histology (72%).
Classification of the cases according to the ECDC case definitions showed that 9.1% (288/3171) of all notified TB cases in The Netherlands during 2007-2009 was '*possible TB'*, 5.7% '*probable TB'* and 71.9% '*confirmed TB'*.

Discussion

The current study provides characteristics of a multi ethnic patient population of 950 non-culture confirmed TB patients in The Netherlands. We found 13 positive culture results in the patient records. These positive cases accounted for 2.5% of the cases in the studied group and overall culture confirmation of notified cases TB cases in 2007-2009 increased only slightly from 70 to 71%. Furthermore, our study shows that although TB diagnosis may seem less robust lacking positive culture, 42% of the diagnoses were actually established with other supportive laboratory evidence. In clinical ETB culture was mostly not performed because biopsy material was put in formalin or no material was obtained during the procedure. In total 63% of possible or probable TB cases were ETB cases. Possible TB cases were more often children, and diagnosed by PHMS physicians in the context of active case finding, and culture was not attempted in these cases. Probable TB cases were more often culture negative, diagnosed by clinicians. The reason of not culturing in this group was more often wrong preservation of the body materials.
One third of the non-culture confirmed cases represented cases traced in active case finding by physicians of the PMHS. Active case finding is a policy propagated to interrupt the chain of transmission in low incidence countries[12] and performed in The Netherlands by the PMHS. The presumed likelihood of infection combined with clinical features of active TB do not always prompt these TB physicians to seek further culture confirmation through invasive procedures.

Table 3. *Characteristics of patients with possible, probable and confirmed TB diagnoses*

Variable, n= (%)		Possible TB N = 288	(%)	Probable TB N = 180	(%)
Diagnosis	PTB ETB P + E	83 182 23	29 63 8	47 113 20	26 63 11
Age groups	0-14 15-44 45-64 >65	89 129 42 28	31 45 15 10	2 92 49 37	1 51 27 21
Foreign born	No Yes Missing	146 141 1	51 49	121 59	67 33
Other diagnoses excluded#	No Yes Missing	214 71 3	74 25	117 62 1	65 34
Culture	Not done Performed Unknown	196 88 4	68 31 1	70 109 2	39 61 1
Reason not cultured	Not sampled No material obtained Not to lab Wrong conditions	175 11 5 4	88 6 3 1	4 4 7 57	6 6 10 79
Diagnosis made by	PMHS Pulmonologist Pediatrician Others Missing	169 90 7 20 2	59 31 2 7	14 131 3 30 2	8 73 2 17
Active case finding	yes No Unknown	147 131 10	51 45 4	13 148 19	7 82 11
TB Symptoms	No Yes Missing	96 190 2	33 66	0 179 (99) 1	0 99
Radiology	No Yes Missing	60 227 1	21 79	57 122 1	31 68
Response to treatment*	No Yes Missing	35 223 30	12 77 10	43 111 26	24 62 14

*Response to treatment favorable: clinically and/or radiologically
#Other diagnosis excluded: when clear from the medical history or correspondence from the hospital

Their specific professional background may cause some extent of diagnostic bias when faced with symptoms resembling active TB or risk groups prone to TB. This policy does not comply with the recently published European Union standards for TB care to submit at least two sputum specimens for microscopic examination, culture and drug susceptibility testing in case of suspicion of PTB. If ETB is suspected, appropriate specimens from the site of involvement should be obtained for examination.[13] However, this recommendation may jeopardize early diagnosis. In contact tracing, especially in young children, early diagnosis may considerably prevent morbidity. Moreover, in case of a low bacterial load, as generally observed in ETB,[6] the results of invasive diagnostics may be disappointing, and frequently do not outweigh the patient burden, especially in children. The majority of all '*possible TB*' cases in this study responded favorable to treatment. This parameter is used as a surrogate marker for disease confirmation by clinicians in case of high clinical TB suspicion and is used after two months of treatment to decide whether to continue treatment or not.[14]
Over sixty percent of cases classified as '*probable TB*' were cultured and TB diagnosis was based on positive results of other laboratory tests. ZN results of body materials other than sputum are not captured in the current national and international surveillance registries. Nonetheless, this is important information to assess the quality of diagnosis.

Unfortunately, in one third of the cases in the '*probable TB*' group, material was fixated in formalin and could not be cultured. Probably they were primarily suspected for a malignancy and TB did not feature in the physicians' differential diagnosis list. This can be explained by the declining TB incidence accompanied by a low index of suspicion and declining expertise.[12] However, in the era of increasing incidence of COPD and Non-tuberculous mycobacterial lung diseases[15-16] it will become increasingly important to consider *Mycobacterium* culture of all surgically resected materials.
Second, this study showed that 6% of the notified cases in the NTR (191/3171) culture status was incorrectly classified. The most common misclassification was 'culture not done or results unknown', while this information could be retrieved from the hospital or laboratory files of the patient. The PMHS collect data for the NTR, not only from patients treated by these organizations but also from those treated by other physicians in the covered geographic region. Hospital clinicians and laboratories lack awareness about the relevance of all laboratory results, including negative results for national TB surveillance.[17]

The scientific board of respiratory disease specialists in The Netherlands has recently adapted the idea of installing TB coordinators in every hospital. Beside clinical tasks and a role in prevention of nosocomial transmission, they can also be informed on the importance of a correct registration in refresher courses organized by the scientific board on a regular basis. Residents in respiratory diseases have four days of compulsory TB education on a national basis and their responsibilities concerning NTR data supply should have more attention in the future. Standard questionnaires may help the clinicians to supply all the necessary information to the PMHS in an efficient way.
Advanced training of PMHS staff in registration methods may further improve the quality of the NTR. After correction of the notification data, the percentage of culture confirmed cases slightly increased to 70.9%. The lower culture confirmation rates in 2007-2009 compared to earlier periods is most likely explained by the increase in the proportion of cases with ETB from 38% to 44% in this period.[2,10]

These ETB cases had a low rate of culture confirmation of 58% compared to PTB cases (79%). Data registration, surveillance methods, case definitions and clinical practice did not change during this period.
Data concerning laboratory samples exchanges and other errors, or culture contamination were studied but could not be extracted systematically from the PMHS patient records, nor from the information supplied by the clinicians or laboratories. We conclude the quality of diagnosis through culture confirmation in The Netherlands can be considered high among PTB patients in comparison with other low incidence countries[18] and is comparatively low in patients with isolated ETB for reasons mentioned above. In 2010 and 2011, the culture confirmation rates among notified TB cases increased again to 74 and 72% respectively, so there is no reason to question the quality of microbiology in our laboratories.
Our study has a few limitations. First, we have only validated a small random sample of the adult culture negative cases from four different PMHS. We learned however that 91% of the results in the NTR of these cases was confirmed in our study. Second limitation is the lack of earlier validation studies in our country. We are therefore not able to detect a trend in the quality of TB diagnosis in The Netherlands.

Conclusions and recommendations: In terms of the quality of the registered data on culture, we found that 'culture negative' results were accurate in 91% of the cases, but 'missing' or 'unknown results' could mostly be extracted from the patient files or the laboratory registries. However, only few cases did have a positive culture and the overall culture confirmation rate did not really change after correction of incorrect or missing data.
In terms of patient characteristics, we found that TB patients with non-culture confirmed disease were more often those with paucibacillary disease.
In terms of the quality of diagnosis we found that most 'possible' TB cases were those with a high likelihood of infection, diagnosed through active case finding by physicians of the PMHS. In the large majority of cases, no material was retrieved for culture. 'Probable' TB cases were mostly diagnosed in the hospital, culture was attempted in 60% of the cases but remained negative. In 31% of the cases the body material was retrieved, but delivered in the wrong condition to be cultured.

Clinicians in The Netherlands, should be aware of the increasing need to culture biopsy and resection material with the increasing incidence of drug resistant TB and NTM infections. TB physicians should be aware of the risk of false positive diagnosis of TB disease in active case finding even if the likelihood of infection is high. At least 2 attempts to obtain a sputum sample or other body material should be made according to the European Standards of TB diagnosis, without endangering the opportunity for early diagnosis and treatment.
Increased awareness of hospital clinicians and laboratories to provide information on all relevant laboratory diagnostic results will improve registration and surveillance to serve TB control. Standard questionnaires may help clinicians to supply all the details needed to the PMHS to complete all data for the NTR. Advanced training of PMHS staff may further improve quality of the registered data in the NTR.

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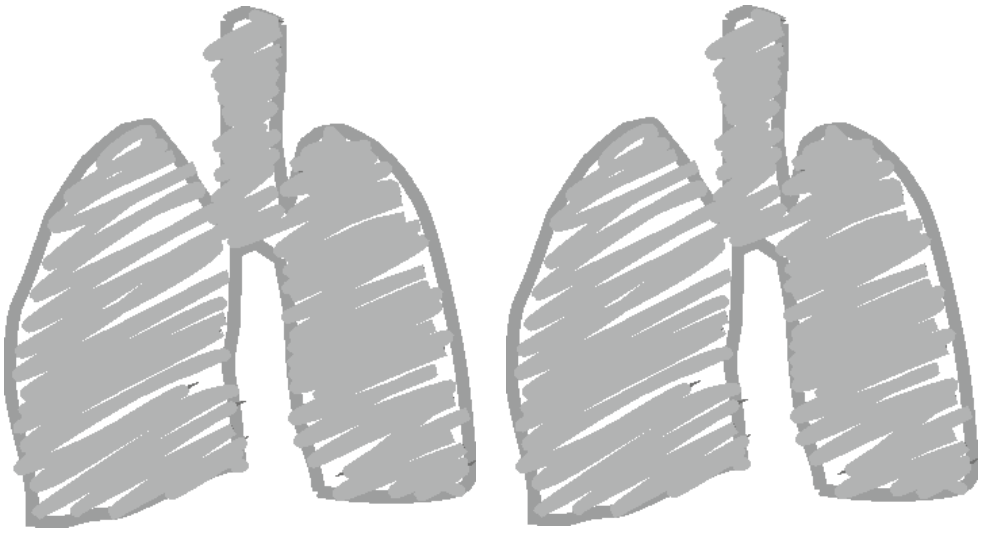
Clinical characteristics and diagnostic delay in spinal tuberculosis patients in The Netherlands

Chapter 5



Abstract

With declining TB incidences diagnostic delays may increase. We describe the patient and clinical characteristics of spinal TB and assessed the course of diagnostic delays from 2000-2011 in The Netherlands. Data from the Netherlands Tuberculosis Registry were studied, completed with from the patient records at the public municipal health services.
A total of 274 cases were studied. Median diagnostic delay was five months during 2000-2011. Sex and age groups were associated with significant differences in diagnostic delay.

No difference was observed between origin of patients, patients presenting with TB risk factors or with neurological symptoms. Typical TB symptoms at presentation lead to significantly increased doctors' delay (typical symptoms 4.0 vs no typical symptoms 2.0 months, p=0.05). Considering spinal TB diagnosis and act expeditious is necessary to limit the time to diagnosis in spinal TB. Refresher courses should be offered both to family physicians and clinical specialists in The Netherlands.

Introduction

Worldwide, tuberculosis (TB) remains a great healthcare problem. In 2010, 8.8 million TB cases were registered with 1.45 million deaths.[1] TB is the second leading cause of death from infectious diseases after HIV/AIDS.[2] Extrapulmonary TB accounts for 1-5% of all TB cases. In turn, bone- and joint tuberculosis (BJTB) comprises 15-35% of all extrapulmonary TB, of which 50 percent is localized in the spine.[3-8] Spinal TB was first described by Percivall Pott in 1779 (Pott's Disease).[9] Treatment of spinal TB consists of chemotherapy with at least isoniazid, rifampicin and pyrazinamide in case the infection is caused by normal susceptible *M. tuberculosis* complex. Six months of treatment is recommended in BJTB by the American, British and Dutch TB guidelines.[10-13] In selected cases chemotherapy should be combined with surgery.[7-8,14-15]
In The Netherlands, TB incidence is declining, with 1443 reported cases in 2000 and 1073 in 2010.[16] As a consequence, patient characteristics and clinical symptoms of (extrapulmonary) TB become less well known. For this reason TB is no longer high in the list of differential diagnosis and this may lead to prolonged diagnostic delay.
In this study we aimed to describe the patient- and clinical characteristics of patients with spinal TB and to assess its course of diagnostic delay in The Netherlands from 2000-2011.

Study population and Methods

In The Netherlands TB is a notifiable disease. For this purpose TB cases are registered in the Netherlands Tuberculosis Registry (NTR). Detailed information is collected and registered on a voluntary basis by the Public Municipal Health Services (PMHS), but contains data of virtually all TB patients in The Netherlands. A retrospective, descriptive study was conducted based on the NTR data in the period 2000-2011. Patients registered with BJTB were selected and all spinal TB cases were evaluated. To study the individual case records, prior permission was needed from the physicians of the PMHS. Basic demographic data, clinical presentation, localization in the spine, TB risk factors, diagnostic methods, and therapy were recorded. As to diagnostic methods, spinal TB was considered confirmed in patients who were diagnosed by culture or by positive ZN as well as PCR results, according to the European Centre for Disease Prevention and Control (ECDC) criteria.[17]

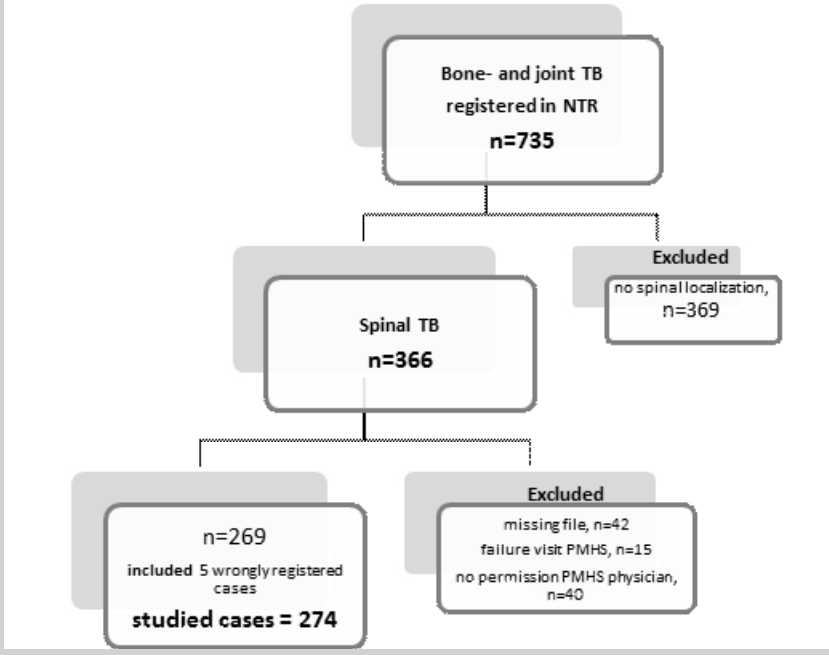
Patients' delay was defined as the time from initial onset of symptoms to the first consultation to a physician. Doctors' delay was defined as the time from the first consultation to the time of diagnosis. Diagnostic delay was defined as the time from the moment symptoms developed to the time diagnosis was established. Time to diagnosis is strictly not always entirely delay; we have used this definition to be consistent with the use in the general literature. Statistical analysis was performed in SPSS, version 18.0. Basic demographic data, clinical presentation, localization in the spine, TB risk factors, diagnostic methods, and therapeutic interventions were presented descriptively. Patients', doctors' and diagnostic delay were also described using appriopriate measures of central tendency and spread considering the non-normal distribution of these delays. Delays subdivided according to patient characteristics or possible other determinants were described as we

were primarily interested in clinically relevant (long) differences in delay. They were also tested using the Wilcoxon rank sum test, Kruskall Wallis one-way ANOVA or correlation analysis (Spearman correlation coefficient) dependent on the determinant and without correction for multiple testing. Determinants evaluated were sex, age group, foreign born, presence of any risk factors, symptoms, 'trias of symptoms' typical for spinal TB, and neurologic deficit at presentation.

Results

From 2000-2011, a total of 14,382 TB patients were registered in the NTR. Seven hundred and thirty five had BJTB (735/14,382 (5%)). Fifty percent of these (366/735) were spinal TB. Three PMHS did not give permission to study the patient records for various reasons, 15 patient records could not be studied due to logistic reasons, and 42 patient records were missing. Five patients were wrongly registered in the NTR. A total of two hundred and seventy-four patients were evaluated in this study (figure 1).

Figure 1. Case selection



Patients characteristics

Patients characteristics are summarized in table 1. Two hundred and seventy-four patients with spinal TB were evaluated, 163 males (59%) and 111 females (41%).

Table 1. Patient characteristics

		Total
Sex	Male	163
	Female	111
Age	0-14	3
	15-24	52
	25-34	61
	35-44	60
	45-54	41
	55-64	19
	65-74	24
	>75	14
Origin	Dutch	41
	European	5
	African	155
	Asian	56
	South-American	17
Diagnosing physician	Pulmonologist	91
	Internal medicine	88
	Orthopedics	33
	Neurology	29
	Neurosurgery	10
	Surgery	4
	Other	19

Clinical symptoms and localization of spinal TB. The most frequent presenting symptom was backache (96.7%), followed by weight loss (54.2%), night sweats (39.9%), fever (31.9%), limb weakness and numbness (15.8%), isolated limb weakness (11%) or isolated limb numbness (5.5%). Seven patients (2.6%) had paraplegia, three patients (1.1%) had tetraplegia. 80 patients (29%) presented with a combination of backache, weight loss and night sweats. Affected spinal sites are summarized in table 2. Nineteen percent of the patients had more than one affected site (53/274). The thoracic spine was affected most (134), followed by lumbar (120), cervical (49) and sacral sites (28).

Table 2. Spinal localizations and treatment

		Treatment			**Total**
		Conservative (%)	Surgery (%)	Unknown	
Involved spinal sites	One	124 (65)	64 (34)	2	190
	More	34 (64)	19 (36)	0	53
	Unknown	19 (61)	6 (19)	6	31
Localization in the spine*	Cervical	32 (65)	17 (35)	0	49
	Thoracic	78 (58)	54 (40)	2	134
	Lumbar	83 (69)	36 (30)	1	120
	Sacral	24 (86)	4 (14)	0	28

***** Total > 274 as 53 patients have more than one affected site

TB risk factors. Seventy-five patients (27%) had risk factors for TB and some had more than one. Type 2 Diabetes Mellitus (n=23), known TB contacts (21), pregnancy (14), malignancy (11), intravenous drug abuse (n=5), HIV-infection (n=4), BCG-treatment for bladder cancer (2), renal failure (2), surgery (2) and alcohol abuse (1) were risk factors found in the studied group.

Diagnostics and treatment. 157 patients initially visited a general physician. From 104 patients this information could not be retrieved from the patient record. Diagnosis was made by specialists in respiratory disease (33.2%), internal medicine (32.1%) and others (34% : neurology, (orthopedic-/neuro-) surgery and others. All patients met the clinical criteria of TB, defined as signs, symptoms and/or radiological findings consistent with active TB. In addition, some had positive acid-fast bacilli detected, or had detection of Mycobacterium Tuberculosis (MTB) in nucleic acid or granulomas in histology. Diagnosis was confirmed by a positive culture in 196 patients (72%). Sixty-two patients (23%) were treated based on clinical and radiological symptoms. Sixteen had positive PCR results or histological evidence for TB (5.8%) or histological evidence for TB (4.0%). 171 cases had normal susceptibility for all first-line drugs (isoniazid, rifampicin, pyrazinamide and ethambutol). 19 cases had mono-resistant TB, 6 had multi drugresistant (MDR) TB. In 78 cases drug susceptibility could not be determined due to negative cultures or to the fact that no culture was performed.

Treatment. All patients were treated by a physician with a full (at least 6 months) course of anti-TB therapy. 264 patients completed treatment. 6 patients died before finishing treatment, none directly related to TB. 4 patients were lost to follow-up. Patients with cervical lesions underwent surgery in 35%, with thoracic lesions in 40%, with lumbar lesions in 30% and with sacral lesions in 14% of the cases. Patients presenting with spinal lesions localized in one site underwent surgery in 34% (64/190) of the cases. Patients with multiple lesions underwent surgery in 36% (19/53) of the cases.

Patients'delay, doctors'delay, diagnostic delay and its determinants. Patients delay could be extracted from the patient record in 142 out of 274 cases (52%). Median patient delay was 1.0 month (range 0-72). Patients delay showed a slight decrease during our study period (figure 2). In 164 out of 274 cases (60%) we were able to define doctors delay from the patient records. Median doctors delay was 3.0 months (range 0-52). Finally , from 197 out of 274 patient records(72%) we were able to determine diagnostic

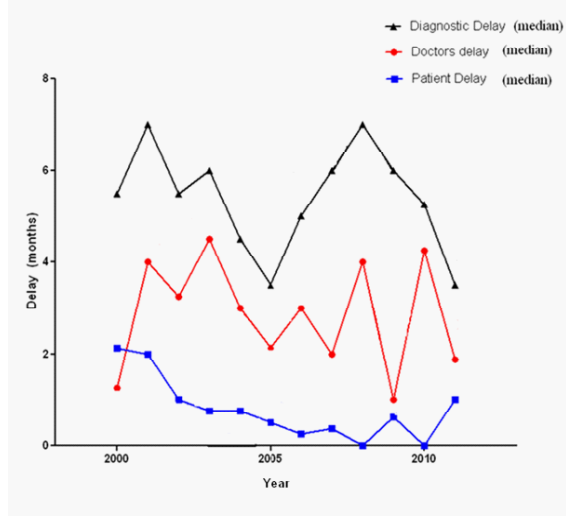


Figure 2. Course of the patients delay, doctors delay and diagnostic delay through the years.

delay. Median delay was 5.0 months (range 0-76). Twenty nine patients had a diagnostic delay longer than 12 months. Diagnostic delay appeared to be constant over the years, possibly showing a slight decrease in recent years (figure 2). Patient characteristics and determinants and their association with patients'delay, doctors'delay and diagnostic delay are shown in table 3.

Table 3. Determinants of delay

Determinant	**value**	**Patients' delay** Median in months (min-max)		**Doctors' delay** Median in months (min-max)		**Diagnostic delay** Median in months (min-max)	
Sex*	**M**	0.75 (0-37.5)	P=0.139	2.25 (0-24)	P=0.052	4.5 (0.25-38.5)	P=0.004
	F	1.00 (0-72)		4.00 (0-52)		5.5 (0-76)	
Age#	**0-34 ys**	0.75 (0-9)	P=0.235	2.00 (0-52)	P=0.022	4.5 (0.25-52)	P=0.006
	35-64 ys	1.00 (0-72)		4.00 (0.25-27)		5.75 (0.75-76)	
	> 65 ys	1.50 (0-18)		3.25 (0-24)		5.00 (0-25)	
Origin*	**NL**	1.5 (0-12)	P=0.883	2.5 (0-24)	P=0.370	5.0 (0-30)	P=0.665
	Fb¶	1.0 (0-72)		3.0 (0-52)		5.0 (0.25-76)	
Risk factors*	**Yes**	1.0 (0-72)	P=0.967	3.0 (0-12.5)	P=0.581	5.0 0.75-46)	P=0.889
	No	1.0 (0-37.5)		3.0 (0-52)		5.25 (0-52)	
Trias of symptoms*	**Yes**	0.5 (0-35)	P=0.483	4.0 (0-27)	P=0.050	5.50 (0.5-46)	P=0.582
	No	1.0 (0-72)		2.0 (0-24)		5.0 (0-76)	
Neurol sympt*	**Yes**	0.88 (0-18)	P=0.049	3.75 (0.25-24)	P=0.403	4.25 (0.25-24)	P=0.070
	No	1.0 (0-72)		2.25 (0-13)		5.5 (0-76)	

*Mann-Whitney test, #Kruskal Wallis test, ¶Fb = foreign born, Trias of symptoms = night sweats, weight loss and back pain,
Neurol sympt = neurological symptoms at presentation

Focussing on doctors' delay, we learn from this table that female sex is associated with a clinically relevant longer doctors' and diagnostic delays (resp. median 2.25 vs 4.0 months and 4.5 vs 5.5 months). Another feature is that middle-aged patients (35-64 years) appear to show more doctors' and diagnostic delay than younger patients. Of note, we did not observe an association between the occurrence of risk factors for TB, including origin of the patient, neurological symptoms at presentation and doctors' delay. The typical 'trias of symptoms' for spinal TB (night sweats, weight loss and backache) was even associated with longer doctors' delay.

Discussion

In this study we described patient and clinical characteristics of all patients with spinal TB in The Netherlands. In addition we assessed patients', doctors' and diagnostic delay in this population. We found a median diagnostic delay in spinal TB of five months which remained constant during the studied period . Sex and age groups were associated with significant differences in doctors' and diagnostic delay. No difference in delays were observed between foreign born patients and patients originated from The Netherlands; patients with TB risk factors did not have shorter doctors' or diagnostic delay; and presentation with neurological symptoms did not lead to shorter doctors' delay. Even more surprisingly, typical TB symptoms (back pain, night sweats and weight loss) at presentation lead to significantly increased doctors delay.

Diagnostic delay may lead to increased morbidity, such as kyphotic deformities of the spine, eventually leading to cosmetic problems, ventilatory compromise and paralysis as was shown in previous studies.[3,18-19] Only few studies of diagnostic delay of spinal TB have been performed in low incidence countries.[5,20-22] Jutte et al. found a mean diagnostic delay of 203 days for spinal TB[22] during 1993-2000. In our study, we found a median diagnostic delay of five months, which is hard to compare as we used the more appropriate measure of central tendency. Our mean diagnostic delay was 7.9 months, showing a diagnostic delay roughly similar to the previous period in The Netherlands. Patients delay seemed to decline during 2000 to 2011(figure 2). We were unable to determine whether this is by chance or a true drop. Whether this decline could be explained by improved accessibility to health care of risk groups as immigrants, illegal or substance abusers could not be defined.

Night sweats and weight loss are symptoms considered classical constitutional symptoms for TB but are frequently lacking in patients with (extrapulmonary) spinal TB. In a patient presenting with constitutional symptoms a physician should consider TB as a diagnosis. In our study, we found that patients presenting with night sweats, weight loss and back pain had clinically relevant longer doctors' delay than patients not presenting with this 'trias'. Features associated with spinal TB include prolonged symptomatic course, absence of fever or chills, spinal angulation, neurological defects, and paraspinous masses. Different regions of the spine are preferentially involved in different age groups and usually two vertebrae are involved, either contiguous or in a skip fashion. Classically, there is more extensive destruction of the ventral portion of the vertebrae, which results in anterior wedging as the bone collapses.[23] The distinction in imaging studies between TB and other diagnosis as malignancies of the spine can be difficult.[24] Diagnosis of spinal TB optimally entails recovery of the pathogen from the suspected site by (CT guided) needle biopsy showing caseating granulomas and yield Mycobacteria on culture.

Sex and age groups were associated with significant differences in delay. As to the effect of age, 30-40% of the Dutch population, between the ages of 45-63 years, visit their family physician with complaints of backache.[25] Their pain is mostly attributed to other causes than TB and this may explain why patients in our study, aged 45-55 years, had significantly longer diagnostic delays.

The clinically relevant difference of doctors' and diagnostic delay between men and women is striking to occur in a developed country, but already a known phenomenon in developing countries with a high incidence of TB. Lower access to diagnosis and treatment, biological difference in incidence, and lower sputum smear sensitivity for women compared to men

have been reported as possible explanations in developing countries for the difference in diagnostic delay in TB.[26-28] In general, sex and gender differences are widely observed in medicine and are increasingly recognized as important interventional targets to further improve quality of healthcare.[29]

Foreign born origin and the presence of TB risk factors were not associated with shorter delays. This is undesirable and more attention to continuous education should be paid to prevent late diagnoses and unnecessary morbidity among patients having typical clinical features and characteristics associated with (spinal) TB. Excellent initiatives to increase the awareness of family physicians to immigrant diseases in The Netherlands already exist and should be promoted continuously.[30]
This study has limitations. Patients- and doctors delay was registered in the NTR until 2005. Consequently, the PMHS probably did collect less data on the issue resulting in less accurate findings from this year. Besides this data collection issue, the reported duration of symptoms is always based on a patients' recall and interpretation. Recall bias is thus a threat to the estimates of patients' delay. Second, due to the retrospective nature of our study, there was many missing data.

Conclusion
Considering spinal TB diagnosis and act expeditious is necessary to limit the time to diagnosis in spinal TB. Refresher courses should be offered both to family physicians and clinical specialists in the era of declining TB incidence in order to raise the awareness and knowledge of TB. Hospital TB coordinators may play a crucial role in education to maintain TB expertise among specialists in their hospitals and participate in clinical decision making in difficult diagnostic cases.

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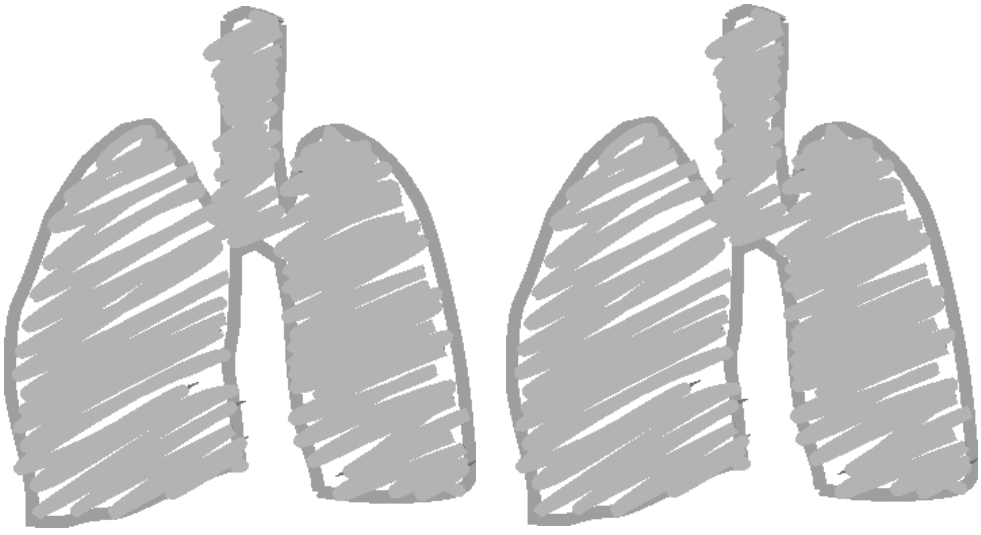
Population pharmacokinetics and limited sampling strategy for first-line tuberculosis drugs and moxifloxacin

Chapter 6



Abstract

Therapeutic drug monitoring (TDM) of tuberculosis (TB) drugs currently focuses on peak plasma concentrations, yet total exposure (AUC_{0-24}) is probably most relevant to the efficacy of these drugs. Therefore we assessed population AUC_{0-24} data for all four first-line TB drugs and moxifloxacin, and developed limited sampling strategies to estimate AUC_{0-24} values conveniently.

AUC_{0-24} and other pharmacokinetic parameters were determined following intensive pharmacokinetic sampling in two Dutch TB referral centers. Best subset selection multiple linear regression was performed to derive limited sampling equations. Median percentage prediction error and median absolute percentage prediction error were calculated via jackknife analysis to evaluate bias and imprecision of the predictions.

Geometric mean AUC_{0-24} values for rifampicin, isoniazid, pyrazinamide, ethambutol and moxifloxacin were 41.1, 15.2, 380, 25.5 and 33.6 h*mg/L respectively. Limited sampling at various fixed sampling points enabled an accurate and precise prediction of AUC_{0-24} values of all drugs separately and simultaneously.

In the absence of clinically validated target values for AUC_{0-24}, the average AUC_{0-24} values could be used as reference values in TDM. Limited sampling of AUC_{0-24} is feasible and allows for TDM at a larger scale in Europe.

Introduction

In the European Union and the European Economic Area (EU/EEA) tuberculosis (TB) cure rates are frequently disappointing, especially in countries where drug resistant TB now poses public health threats.[1] The long duration of TB treatment and adverse events often lead to non-adherence, which is thought to cause treatment failure, relapse and emergence of drug resistance.[2] Besides this, several studies on relationships between drug exposure and response,[3-5] results from the *in vitro* hollow fibre model,[6] and findings from a recent meta-analysis,[7] point to pharmacokinetic variability as an additional culprit of treatment failure and acquired drug resistance. Unfortunately, the determinants associated with the variability in drug exposure are not entirely clear and predicting low exposure in TB patients remains difficult.[8]

In this context therapeutic drug monitoring (TDM), i.e. measurement of plasma concentrations and individualization of drug dosing based on these measurements, seems useful to improve treatment response. Our group and others have previously described that TB drugs fulfill several but not all criteria for TDM. Indications for TDM in selected patients have been formulated, and experience in clinical practice shows that this tool can be of decisive value in individual patients.[3,9-12] As a result, the practice of TDM is already accepted in some treatment centers, yet it is rejected in others in view of remaining prospective evidence to be gathered. Still, it is surprising that the recently published European standards for TB care developed by the European Centre for Disease Prevention and Control (ECDC) / European Respiratory Society (ERS) task force did not mention pharmacokinetic evaluation as a method of patient-centered approach at all.[13]

Unfortunately, clinically validated targets for measures of exposure to TB drugs are unknown. However, reference ranges for peak plasma concentrations (C_{max}) of TB drugs have been described in the past, representing the normal concentrations that can be expected in adults with standard doses of TB drugs.[9] Using these C_{max} reference values, TDM for TB drugs involves sampling early in the pharmacokinetic curve as a means to 'catch' and estimate the C_{max} concentration in an individual, as described by Peloquin[9] and applied by others.[10-12] Evolving data however suggest that the total exposure to TB drugs or the 'area under the concentration versus time curve' (AUC_{0-24}) is more relevant to the efficacy of the first-line TB drugs[14-19] and of moxifloxacin, a second line drug that is being evaluated as a first-line TB drug.[20] Ideally, AUC_{0-24} is combined with the minimum inhibitory concentration (MIC) of the mycobacteria, to yield AUC_{0-24}/MIC that is supposed to best predict response. To enable forecasting of drug dosing based on total exposure, reference population pharmacokinetic data concerning the AUC_{0-24} are needed. In addition, a limited (or optimal) sampling strategy is required, using a limited number of concentrations measured at predefined times after dosing in order to estimate AUC_{0-24} in an individual.[21]
The alternative of recording a full pharmacokinetic curve is often not feasible in patient care, as it implies a significant patient burden, a large time investment to collect samples, and high costs. Therefore the objective of this study was to assess the population pharmacokinetics of rifampicin, isoniazid, pyrazinamide, ethambutol and moxifloxacin in patients from TB referral centres in The Netherlands and to develop limited sampling strategies to estimate the AUC_{0-24} of these drugs.

Methods

Subjects. The study subjects were TB patients admitted to the two Dutch TB referral centres, namely University Medical Centre Nijmegen, Centre for Chronic Diseases Dekkerswald, Groesbeek and University Medical Centre Groningen, Tuberculosis Centre Beatrixoord, Haren. TB was diagnosed by the gold standard (positive culture of *Mycobacterium tuberculosis complex*). Patients were included if at least 18 years of age and they had to provide written informed consent. The study protocol was approved by the Ethical Review Board of University Medical Centre Nijmegen, The Netherlands. A local feasibility declaration was obtained from University Medical Centre Groningen as required by Dutch law.

Design and procedures. A descriptive pharmacokinetic study was performed. Pharmacokinetic parameters were generated using the standard two-stage approach. With this approach, individual pharmacokinetic parameters are estimated in the first stage. In the second stage, population characteristics of each parameter are derived by obtaining measures of central tendency and spread of all the subjects' individual parameters. Patients were recruited consecutively. TB drugs were administered once daily and dosed according to the Dutch Society of Pulmonary Diseases and Tuberculosis (NVALT) guidelines for medical TB treatment.[22] TB drugs were taken simultaneously, under supervision of a nurse. All TB drugs were approved and separately formulated. Other (concomitant) medication was taken at least four hours after TB drug intake. A full pharmacokinetic curve was recorded during the intensive phase of TB treatment after steady state was reached (≥ 2 weeks). Patients refrained from food intake from 11.00 p.m. on the day preceding the pharmacokinetic assessment until four hours after intake of study medication. Serial venous blood samples were collected just prior to, and at 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12.0 and 24.0 hours after witnessed intake of study medication. Plasma was separated immediately, frozen to -80°C and transported on dry ice for bio-analysis.

Bio-analysis. Plasma concentrations of rifampicin, pyrazinamide, ethambutol and moxifloxacin were assessed at University Medical Centre Nijmegen, using validated high performance liquid chromatography (HPLC) methods as described before.[23-24] Plasma concentrations of isoniazid were determined at the University Medical Centre Groningen with a validated method using protein precipitation followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). This bio-analytical method for isoniazid has not been described before and details can be read in the appendix of this article.

Pharmacokinetic analysis. Pharmacokinetic analysis was performed with non-compartmental methods using WinNonLin version 5.0 (Pharsight Corp., Mountain View, California) as previously described.[23] Most importantly, AUC_{0-24} was calculated using the linear-log trapezoidal rule. If the concentration at 24 h post dose (C_{24}) was below the limit of quantitation (for rifampicin and isoniazid) it was calculated using the formula $C_{24} = C_{last} * e^{-\beta*(24-Tlast)}$, in which β is the first order elimination rate constant and C_{last} is the last measurable concentration sampled at T_{max}. β was obtained by least squares linear regression analysis on log C versus time, with the absolute slope of the regression line being β/2.303.

Statistics. Descriptive statistics were calculated for each of the pharmacokinetic parameters. For each TB drug, the proportion of patients with C_{max} within reference values was assessed. Reference C_{max} values of the first line TB drugs were as follows: rifampicin 8-24 mg/L, isoniazid 3-6 mg/L, ethambutol 2-6 mg/L and pyrazinamide 20-50 mg/L[9] or ≥35 mg/L(5). These reference C_{max} values are based on data that were compiled from all available sources (both healthy volunteers and TB patients) by, amongst others, Holdiness and Peloquin.[25-26] For moxifloxacin, standard minimum inhibitory concentration (MIC) values of 0.25 and 0.5 mg/L were used to estimate the pharmacodynamic indices AUC_{0-24}/MIC and C_{max}/MIC. These ratios should reach the desired values of 100 and 10, respectively, to achieve the greatest bactericidal effect and a decreased probability of resistance, at least when moxifloxacin is used against fast-growing Gram negative bacilli.[20]
Pharmacokinetic parameters were log transformed before further statistical analyses. Comparison of pharmacokinetic parameters between subgroups was performed using the independent sample t-test on the log-transformed parameters or the Wilcoxon rank-sum test for time to C_{max} (T_{max}). Correlations between numerical variables were calculated using the non-parametric Spearman's rank correlation coefficient (rho).

Best subset selection multiple linear regression was performed to derive limited sampling equations predictive of the AUC_{0-24} of individual TB drugs, and for simultaneous prediction of the AUC_{0-24} of rifampicin, isoniazid, pyrazinamide, ethambutol and moxifloxacin. To maintain clinical applicability, all models were constrained to include three samples at the most, collected within 6h after drug administration. For all subsets containing one, two or three samples, we calculated the average adjusted r square and the model with the biggest average r square was chosen. In addition, it was evaluated whether the practice of sampling at 2h, 4h and 6h post dose in order to 'catch' C_{max}, as applied in one of our TB referral centres, provides an accurate estimation of AUC_{0-24}.

To assess the predictive performances of the models we calculated residuals for each patient based on models fitted to a dataset where that patient was omitted (jackknife analysis).[27] Potential bias in the predictions was assessed using the median prediction error (MPE) and median percentage prediction error (MPPE); for this latter measure, residuals were converted to percentages by dividing the residuals by the predicted values. Imprecision was assessed using root median squared prediction error (RMSE) and median absolute percentage prediction error (MAPE). MPPE and MAPE of <15% were considered acceptable.[27] All statistical analyses were performed with SPSS for Windows version 20.0 (SPSS Inc, Chicago, IL) and R version 2.15.2. P values of below 0.05 were considered significant.

Results

Patients. Forty-one TB patients were included in the study. Patient and treatment characteristics are summarized in table 1. Four out of 41 patients were diagnosed with TB caused by mycobacteria that were resistant to isoniazid and rifampicin (multi-drug resistant TB, MDR-TB). Moxifloxacin was used in patients for MDR-TB (n=12), because of possible MDR-TB based on treatment history, and in case of intolerance for other TB drugs. Concomitant medication was not expected to influence the pharmacokinetics of the TB drugs (data not shown). None of the patients had compromised liver or kidney function (defined respectively as transaminases ≥ 50 U/L and glomerular filtration rate < 60 ml/min).

Table 1. Patient characteristics

Characteristic	Value
Demographic characteristics	
Male, n (%)	32 (78)
Age, median (IQR)	42 (27)
Body weight (kg), mean (SD)	64.5 (14.6)
Ethnicity, n (%)	
Caucasian	12 (29)
Black	13 (32)
Asian	5 (12)
Indian-Afghan	5 (12)
Middle-East	4 (10)
Other / mixed origin	2 (5)
Type of TB	
Pulmonary	34
Extrapulmonary	7
Co-morbidity, n(%)	
HIV co-infection	3 (7)
Diabetes mellitus	1 (2)
Dose, mg/kg, median (range)	
Rifampicin	9.3 (4.7-13.0)
Isoniazid	4.5 (2.3-6.1)
Pyrazinamide	28.7 (11.7-32.5)
Ethambutol	20.3 (15.9-26.0)
Moxifloxacin	7.0 (5.7-9.3)

#other co-morbidities are cancer, cardiovascular disease, respiratory diseases

Pharmacokinetics of TB drugs. Table 2 displays the pharmacokinetic parameters of rifampicin, isoniazid, pyrazinamide, ethambutol and moxifloxacin. Remarkable inter-patient variability in total exposure (AUC_{0-24}) was observed for rifampicin (range 4.6-118.9 h*mg/L), isoniazid (6.1-29.8 h*mg/L), ethambutol (13.8-86.4 h*mg/L) and moxifloxacin (15.2-84.2 h*mg/L). Exposure to pyrazinamide showed relatively small inter-patient variability (267-679 h*mg/L).

Table 2. Summary of pharmacokinetic parameters of rifampicin, isoniazid, pyrazinamide, ethambutol and moxifloxacin

Pharmacokinetic parameter* (range)	Rifampicin N=34	Isoniazid N=14	Pyrazinamide N=19	Ethambutol N = 19	Moxifloxacin N=12
AUC_{0-24} (h.mg/L)	41.1 (4.6-118.9)	15.2 (6.1-29.8)	380 (267-679)	23.5 (13.8-86.4)	33.6 (15.2-84.2)
Cmax (mg/L)	9.5 (1.3-24.8)	3.2 (1.3-5.2)	44.5 (33.6-60.3)	3.3 (2.0-7.6)	3.3 (2.4-5.8)
Tmax (h)	2.0 (1.0-6.0)	1.3 (1.0-2.5)	1.5 (1.0-2.5)	3.0 (1.0-6.2)	2.0 (1.0-4.0)
Cl/F (L/h)	14.5 (5.0-129.6)	19.8 (10.1-49.3)	4.6 (2.6-6.3)	54.3 (16.2-87.3)	11.9 (4.8-26.3)
Vd/F (L)	39.2 (17.8-379.1)	82.1 (49.3-187.9)	37.0 (27.5-55.2)	723 (249-1651)	142.3 (89.5-270)
t½(h)	1.9 (1.0-4.4)	2.9 (1.7-4.5)	5.9 (3.0-13.8)	9.2 (6.3-16.0)	8.3 (5.4-13.1)

* Geometric mean and range, apart from tmax, for which median and range are displayed.
AUC0-24: area under the concentration versus time curve, Cmax: peak plasma concentration, Tmax: time to peak plasma concentration, Cl/F: apparent clearance, Vd/F: apparent volume of distribution, t1/2: elimination half-life.

One third of patients had low peak plasma concentrations of rifampicin (<8 mg/L, 10/33 patients) and isoniazid (<3 mg/L, 4/12). Only one patient had a low C_{max} of pyrazinamide below 35 mg/L but above 20 mg/L. No patients showed low peak plasma concentration of ethambutol. Geometric mean values (range) for moxifloxacin AUC_{0-24}/MIC and C_{max}/MIC were 67.2 (30.4-168.4) and 6.6 (4.8-11.6) respectively. Three out of 12 patients had moxifloxacin AUC_{0-24}/MIC above 100 based on a fixed MIC of 0.5 mg/L. Based on a fixed MIC of 0.25 mg/L, 10/12 patients would have moxifloxacin AUC_{0-24}/MIC above 100.
The geometric mean AUC_{0-24} of moxifloxacin was 28.1 h*mg/L when combined with rifampicin (n=5) compared to 38.1 h*mg/L for moxifloxacin without rifampicin (n=7) (p =0.172).
No clear associations were found between pharmacokinetic parameters and age, gender or BMI. Only for rifampicin, there was a trend to lower AUC_{0-24} with increasing weight (Spearman's rho -0.30, p = 0.08). The number of patients with HIV/AIDS and diabetes mellitus and numbers of patients within various ethnic groups were considered too small to test.
Limited sampling equations. Table 3 shows the best performing one, two and three time point equations of all four first-line TB drugs and moxifloxacin separately when administered on an empty stomach. For pyrazinamide, ethambutol and moxifloxacin a high

adjusted R^2 (>0.90) was already reached with only one sample at 6 h post dose, but a set of three samples clearly gave the highest adjusted R^2 (table 3). For rifampicin, sampling at 1, 4 and 6 h post dose enabled the best prediction of AUC_{0-24}. For isoniazid, samples at 1, 2.5 and 6 h post dose were best. Sampling at 0, 2 and 6 h post dose for pyrazinamide; at 0, 2.5 and 6 h post dose for ethambutol; and at 0, 1.5 and 6 h post dose for moxifloxacin enabled the best prediction of exposure (table 3).

Table 3. *Best performing one, two and three sampling point strategies for estimation of AUC_{0-24} of individual TB drugs* *****

Sampling times		Equation	Adjusted R^2	MPE	MPPE %	RMSE	MAPE%
RIF	C_4	$AUC_{0-24} = -0.86 + 7.16 \times C_4$	0.8365	-1.72	-3.63	7.09	16.07
	C_2, C_6	$AUC_{0-24} = 0.57 + 2.68 \times C_2 + 6.98 \times C_6$	0.9321	-0.40	-1.08	2.99	8.49
	C_1, C_4, C_6	$AUC_{0-24} = -4.71 + 1.74 \times C_1 + 3.76 \times C_4 + 4.83 \times C_6$	0.9691	-0.13	-0.15	3.44	7.60
INH	C_3	$AUC_{0-24} = -1.15 + 7.97 \times C_3$	0.8886	-0.46	-2.93	1.35	7.27
	C_4, C_6	$AUC_{0-24} = -0.59 + 5.06 \times C_4 + 7.14 \times C_6$	0.9638	0.45	3.09	0.84	4.91
	$C_1, C_{2.5}, C_6$	$AUC_{0-24} = -1.02 + 2.06 \times C_1 + 1.98 \times C_{2.5} + 6.03 \times C_6$	0.9746	-0.31	-1.70	1.29	7.00
PYR	C_6	$AUC_{0-24} = 41.49 + 13.91 \times C_6$	0.9127	-5.13	-1.38	25.00	6.13
	C_4, C_6	$AUC_{0-24} = -30.18 + 5.02 \times C_4 + 10.14 \times C_6$	0.9539	-2.82	-0.56	13.65	3.47
	C_0, C_2, C_6	$AUC_{0-24} = -15.42 + 7.71 \times C_0 + 3.55 \times C_2 + 8.66 \times C_6$	0.9687	0.40	0.10	12.69	3.56
ETH	C_6	$AUC_{0-24} = 8.16 + 9.98 \times C_6$	0.9295	0.10	0.62	3.02	12.80
	C_0, C_6	$AUC_{0-24} = 6.17 + 12.27 \times C_0 + 7.98 \times C_6$	0.9597	0.19	1.10	2.00	8.16
	$C_0, C_{2.5}, C_6$	$AUC_{0-24} = 0.91 + 10.24 \times C_0 + 2.86 \times C_{2.5} + 7.45 \times C_6$	0.9751	0.59	2.43	1.88	11.04
MOXI	C_6	$AUC_{0-24} = -2.54 + 16.35 \times C_6$	0.9608	-0.74	-2.07	3.85	8.94
	$C_0, C_{2.5}$	$AUC_{0-24} = 1.40 + 25.04 \times C_0 + 6.84 \times C_{2.5}$	0.9855	0.65	1.98	1.83	4.30
	$C_0, C_{1.5}, C_6$	$AUC_{0-24} = 1.43 + 16.75 \times C_0 + 2.12 \times C_{1.5} + 8.18 \times C_6$	0.9935	0.58	2.26	1.45	4.69

* Formulas are based on blood sampling in the first 6 h post dose after administration of drugs on an empty stomach. The formulas do not apply to administration of drugs with food.

Abbreviations: RIF = rifampicin, INH = isoniazid, PYR= pyrazinamide, ETH = ethambutol, MOXI = moxifloxacin, MPE = median prediction error, MPPE = median percentage prediction error, RMSE = root median squared prediction error, MAPE = median absolute percentage prediction error

Table 4 shows the best performing one, two and three time point equations for simultaneous prediction of exposure to all five drugs based on blood sampling in the first 6 h. The best limited sampling strategy for the simultaneous prediction of exposure to all five drugs involved the time points 1, 4 and 6 h. Sampling at 2, 4 and 6 h post dose, as can be applied to 'catch' the peak plasma concentrations, also enabled a good prediction of AUC_{0-24} for all drugs (table 4). Again, these equations were based on and are applicable for intake of TB drugs on an empty stomach.

Table 4. *Best performing one, two and three sampling point strategies for estimation of AUC_{0-24} of 5 TB drugs simultaneously* *****

Sampling times		Equation	Adjusted R^2	MPE	MPPE%	RMSE	MAPE%
RIF	C_6	$AUC_{0-24} = 12.76 + 9.92 \times C_6$	0.6484	0.61	1.96	8.41	17.74
INH	C_6	$AUC_{0-24} = 5.54 + 9.94 \times C_6$	0.6968	-0.27	-1.89	2.60	23.56
PYR	C_6	$AUC_{0-24} = 41.49 + 13.91 \times C_6$	0.9127	-5.13	-1.38	25.00	6.13
ETH	C_6	$AUC_{0-24} = 8.16 + 9.98 \times C_6$	0.9295	0.10	0.62	3.02	12.80
MOXI	C_6	$AUC_{0-24} = -2.54 + 16.35 \times C_6$	0.9608	-0.74	-2.07	3.85	8.94
RIF	C_3, C_6	$AUC_{0-24} = -2.14 + 3.63 \times C_3 + 5.91 \times C_6$	0.8941	0.09	0.37	6.21	14.32
INH	C_3, C_6	$AUC_{0-24} = -1.06 + 5.87 \times C_3 + 4.05 \times C_6$	0.9467	0.61	5.60	1.93	10.50
PYR	C_3, C_6	$AUC_{0-24} = -40.03 + 4.53 \times C_3 + 10.48 \times C_6$	0.9505	-5.54	-1.50	11.29	2.73
ETH	C_3, C_6	$AUC_{0-24} = -0.14 + 4.49 \times C_3 + 8.33 \times C_6$	0.9496	1.03	5.98	2.97	15.51
MOXI	C_3, C_6	$AUC_{0-24} = -4.75 + 4.16 \times C_3 + 12.11 \times C_6$	0.9635	-0.39	-1.24	4.15	10.91
RIF	C_1,C_4,C_6	$AUC_{0-24} = -4.71 + 1.74 \times C_1 + 3.76 \times C_4 + 4.83 \times C_6$	0.9691	-0.13	-0.15	3.44	7.60
INH	C_1,C_4,C_6	$AUC_{0-24} = -0.67 + 0.84 \times C_1 + 3.55 \times C_4 + 7.60 \times C_6$	0.9673	0.03	0.16	1.04	5.24
PYR	C_1,C_4,C_6	$AUC_{0-24} = -48.51 + 0.80 \times C_1 + 5.31 \times C_4 + 9.20 \times C_6$	0.9549	-3.46	-0.80	15.08	3.82
ETH	C_1,C_4,C_6	$AUC_{0-24} = 0.37 + 2.23 \times C_1 + 2.26 \times C_4 + 8.52 \times C_6$	0.9514	1.70	9.18	2.81	13.20
MOXI	C_1,C_4,C_6	$AUC_{0-24} = -4.91 + 2.93 \times C_1 - 0.21 \times C_4 + 14.52 \times C_6$	0.9796	0.39	1.70	2.64	7.57
RIF	C_2,C_4,C_6	$AUC_{0-24} = -2.22 + 2.05 \times C_2 + 2.25 \times C_4 + 4.93 \times C_6$	0.9494	-1.07	-1.85	4.22	9.47
INH	C_2,C_4,C_6	$AUC_{0-24} = -1.46 + 1.18 \times C_2 + 4.71 \times C_4 + 5.51 \times C_6$	0.9674	0.46	2.68	1.20	7.30
PYR	C_2,C_4,C_6	$AUC_{0-24} = -48.65 + 1.78 \times C_2 + 3.58 \times C_4 + 9.90 \times C_6$	0.9554	-4.53	-0.90	10.52	2.57
ETH	C_2,C_4,C_6	$AUC_{0-24} = -6.34 + 5.22 \times C_2 + 2.83 \times C_4 + 7.07 \times C_6$	0.9632	-0.43	-2.11	1.75	7.43
MOXI	C_2,C_4,C_6	$AUC_{0-24} = -4.35 + 3.97 \times C_2 - 6.49 \times C_4 + 20.05 \times C_6$	0.9659	0.18	0.64	4.40	11.95

* Formulas are based on blood sampling in the first 6 h post dose after administration of drugs on an empty stomach. The formulas do not apply to administration of drugs with food.

Abbreviations: RIF = rifampicin, INH = isoniazid, PYR= pyrazinamide, ETH = ethambutol, MOXI = moxifloxacin, MPE = median prediction error, MPPE = median percentage prediction error, RMSE = root median squared prediction error, MAPE = median absolute percentage prediction error

Discussion

The current study first provides steady-state population pharmacokinetic data collected in a typical multi-ethnic population of TB patients admitted to TB referral centres in Western-Europe. In the absence of clinically validated target or cut-off values for AUC_{0-24}, these population (average) values for AUC_{0-24} could be used as reference values in TDM, similar to the use of 'normal' C_{max} values of TB drugs.[9] The reasoning is that these average values represent the best case scenario for the standard doses of the drugs. If an individual can achieve such concentrations, then it would appear unlikely that low drug exposure is the primary reason for suboptimal clinical response, and other explanations should be considered.[9]
Second, this study showed a large inter-individual variability in pharmacokinetics of all TB drugs studied, apart from pyrazinamide, which is consistent with data from other populations.[5,8] Many patients had C_{max} of rifampicin or isoniazid below reference values. Similarly, reference values for moxifloxacin AUC_{0-24}/MIC and C_{max}/MIC ratios were not reached in some patients, dependent on the fixed MIC value used. Large inter-individual variability in exposure to just one TB drug may already contribute to treatment failure or drug resistance,[7] whereas multiple low concentrations may further predispose to these events. Inter-individual variability in exposure is a reason and actually a prerequisite for TDM as a means to individualize drug dosing.

Third, linear regression analyses showed that limiting sampling can be used to make an accurate and precise estimation of AUC_{0-24} values for all first-line TB drugs and moxifloxacin separately and simulataneously. With this strategy, the patient burden is significantly lowered, and time investment and costs are reduced compared with the intensive (11 time points) pharmacokinetic evaluation necessary to assess AUC_{0-24} values as performed in this study. Use of two sampling points at t = 3 and 6 h for simultaneous estimation of all AUC_{0-24} values resulted in acceptable R^2 values, yet the predictive performance of the two sample points equations was less favourable in terms of median absolute percentage prediction error (MAPE, table 4). Three sampling points (at t = 1, 4 and 6 h) resulted in the most accurate simultaneous estimation of AUC_{0-24}. We ourselves favour the sampling at t = 2, 4 and 6 h post dose, as this allows for estimation of C_{max} (which is commonly based on sampling at 2h plus 4h or 6 h post dose[9,12]) as well as estimation of AUC_{0-24}. Alternatively, the population pharmacokinetic data from the current study can be used in another approach to assess AUC_{0-24} in the context of TDM, i.e. maximum a posteriori (MAP) Bayesian fitting (28). This technique uses the 'a priori' population pharmacokinetic parameters, the patient's drug dosing information, and measured plasma concentrations to assess 'a posteriori' pharmacokinetic parameters of the patient, including the AUC_{0-24}. Use of the population parameters in this Bayesian estimation approach requires expertise with specific software, but has the advantage that sampling time points do not need to be fixed, as long as exact sampling times are known. Either with limited sampling formulas or with MAP Bayesian fitting, it seems feasible to evaluate AUC_{0-24} of TB drugs and to individualize doses in patients with a higher a priori risk of treatment failure, relapse rates and emergence of drug resistance. Less invasive pharmacokinetic assessment tools may further facilitate TDM of TB drugs, especially in children and in remote areas. For example, small blood spots can be taken with insulin-type needles, dried on filter paper and sent by regular post mail (Dried

Blood Spot technique, DBS).[29] In addition, saliva could be used as a matrix for TDM.[30]
For the near future, it is important to derive clinically validated cut-off values for $AUC_{0\text{-}24h}$ and C_{max} rather than to normalize exposure to population values. In addition, susceptibility of mycobacteria (MIC) should be taken into account to optimize $AUC_{0\text{-}24h}$/MIC.
One limitation of this study relates to its relatively small sample size, typical to a study with intensive pharmacokinetic sampling. The average $AUC_{0\text{-}24}$ data proposed as reference data for TDM may need to be confirmed in a larger population. Furthermore, we did not prospectively validate our limited sampling formulas. Instead we calculated residuals for each patient based on models fitted to a dataset where that patient was omitted (jackknife analysis), an analysis which is a useful and appropriate approach in cases of small sample size.[27] Finally, when it comes to the application of the limited sampling equations in TDM, it must be considered that accumulating evidence shows an association between exposure of single TB drugs in plasma and treatment outcome and emergence of resistance, but the exact relationships need further elucidation.

In summary, low plasma concentrations of pivotal anti TB drugs have recently been appointed as an additional culprit of treatment failure and acquired drug resistance. The current study provides steady-state data for total exposure ($AUC_{0\text{-}24}$) to first-line TB drugs and moxifloxacin. These population $AUC_{0\text{-}24h}$ values could be used as reference values for TDM, a technique which is feasible in the European setting. Limited pharmacokinetic sampling at 1, 4 and 6 h or at 2, 4 and 6 h post dose enables an accurate and precise simultaneous estimation of $AUC_{0\text{-}24}$ values for all these TB drugs. Evaluation of drug exposure with limited sampling and less invasive assessment methods may become an important tool in the near future to optimize treatment results and prevent emergence of resistance.

Appendix. *Bio-analytical method for isoniazid at University Medical Centre Groningen*
Ten microliter of each plasma sample was added to precipitation reagent methanol and acetonitrile 4:21 v/v containing the internal standard. The mixture was vortexed, stored at -20 °C for about 30 min to promote protein precipitation, and consequently centrifuged. From the clear upper layer, 5 microliter was injected on a 100 mm x 2.1 mm C18, 5µm analytical column set at 20 °C. The mobile phase consisted of a gradient made from an aqueous buffer containing ammonium acetate, acetic acid and trifluoroacetic anhydride, water and acetonitrile. The detector was operating in electrospray positive ionization mode and performed selected reaction monitoring (SRM) as scanning mode. The mass parameters of m/z 138.1→121.1 (collision energy 14eV) were measured with a scan width of 0.5 m/z. Accuracy was between 95.6% and 111.3%, depending on the concentration of isoniazid. The intra- and inter assay coefficients of variation were less than 10.5% over the range of 1.5 to 30 mg/L. The lower limit of quantitation was 0.4 mg/L.

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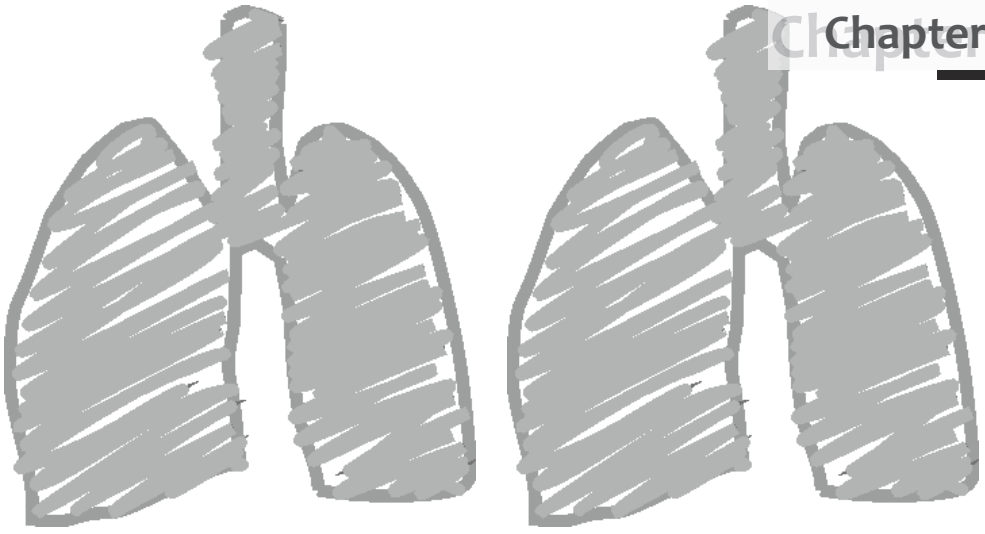
Therapeutic drug monitoring in the treatment of tuberculosis patients

Chapter 7



Abstract

At the University Centre for Chronic Diseases Dekkerswald, a tertiary tuberculosis (TB) referral hospital in The Netherlands, Therapeutic Drug Monitoring (TDM) is used in patients in case of relapse TB, when there is delayed response to TB treatment, and when abnormal TB drug concentrations are suspected for other reasons. In this article, a case series is presented to illustrate the value of individualized TB drug dosing in four patients with low TB drug concentrations. Increased doses of the TB drugs, especially of rifampicin, resulted in adequate peak plasma concentrations and improved clinical response to treatment in these patients, while no adverse events occurred.

Introduction

First-line treatment of drug susceptible tuberculosis (TB) is highly effective. Nevertheless, a number of patients fail to respond to treatment, have relapse TB, or develop drug-resistant TB[1]. Inadequate response to TB treatment results from a complex and poorly understood combination of factors that vary from patient to patient[2].
Suboptimal plasma concentrations of first-line TB drugs, in particular of rifampicin and isoniazid, are among these factors.[3-4] Low plasma concentrations can result from malabsorption in patients with human immunodeficiency virus (HIV) infection or gastrointestinal tract anomalies, diabetes mellitus (DM) as a concomitant disease, or interindividual variability in pharmacokinetics.[5-12]
Therapeutic Drug Monitoring (TDM), i.e. individualized drug dosing guided by drug plasma concentrations,[13] could be of help in improving response to drug treatment. So far, TDM has been applied to TB treatment in a limited number of centres only.[2,10,11,14,15] In our setting, TDM was introduced to a selected group of patients in 2006. Here, we present our TDM practice and a case series that illustrates the value of using TDM in patients with drug-susceptible TB who are suspected to have low TB drug plasma concentrations.

Methods

Setting

The University Centre for Chronic Diseases Dekkerswald is one of two tertiary TB referral hospitals in The Netherlands for patients with severe or complex TB disease, co-morbidities such as HIV-infection, and drug-resistant TB.
TB treatment in this centre is in accordance with guidelines of the Dutch Association of Physicians for Lung Disease and Tuberculosis (NVALT) and the standard, first-line treatment regimen consists of 2 months daily isoniazid (H: 5 mg/kg bodyweight, maximum 300 mg), rifampicin (R: 450 mg in patients with bodyweight < 50 kg; 600 mg in patients with bodyweight > 50 kg), pyrazinamide (Z: 30 mg/kg bodyweight, maximum 2000 mg), and ethambutol (E: 15-20 mg/kg bodyweight, maximum 1600 mg), followed by 4 months of daily H and R, in separate formulations.[16]

TDM practice

In our centre, TDM is used in patients with drug-susceptible TB when abnormal TB drug concentrations are suspected (e.g. in case of HIV-infection, diabetes, gastrointestinal anomalies, malnutrition, or renal or hepatic dysfunction), when there is delayed sputum culture conversion (i.e. after more than 8 weeks of treatment), and in case of relapse TB. Pharmacokinetic sampling is performed after a minimum of 2 weeks of treatment, when steady state concentrations of the first-line TB drugs have been achieved. Samples are taken pre-dose (C0), and at 2 h (C2) after drug intake on an empty stomach to estimate the peak plasma concentration.[10] After initial experiences we currently take samples at 4 (C4) and 6 h (C6) post dose as well, to be able to determine peak plasma concentrations more accurately and to differentiate between delayed absorption and malabsorption. After sampling, plasma is separated immediately, frozen to -80 °C, and transported on dry ice to the laboratory for bioanalysis of TB drugs by validated high-performance liquid chromatographic (HPLC) methods. Previously, plasma concentrations of rifampicin were

routinely measured, considering its known concentration-effect relationship.[10,17] To limit the cost, isoniazid, pyrazinamide and ethambutol concentrations were only determined if the rifampicin concentration was below the lower limit of the target range. Now we measure rifampicin, isoniazid and pyrazinamide at once in all samples.
The following target ranges of peak plasma concentrations are used for first-line TB drugs that are taken in daily, oral doses: 3-5 mg/L for isoniazid, 8-24 mg/L for rifampicin, 20-50 mg/L for pyrazinamide, and 2-6 mg/L for ethambutol.[10] These ranges represent the normal concentrations that can be expected after the standard doses of TB drugs. The ranges are based on data that were compiled from all available sources (both healthy volunteers and TB patients).[5,10] Subsequently the ranges were validated in a range of phase I studies in healthy volunteers.[9] Since then these target values have been adapted in all subsequent pharmacokinetic studies in TB. In our setting, hospital pharmacists interpret the TDM results and respiratory physicians decide on adjusting drug doses.

Results-case series

Patient 1 – relapse TB after previously delayed sputum culture conversion

A 57-year-old Caucasian man with a body mass index (BMI) of 18.6 kg/m^2 and a history of alcohol abuse and smoking was treated with the standard 2HRZE/4HR for susceptible pulmonary TB from January to July 2008. He was on Directly Observed Treatment (DOT) during the first 2 months. Sputum culture conversion occurred late: after 11.5 weeks of treatment. In April 2009, he presented with relapse pulmonary TB (the *Mycobacterium tuberculosis* (MTB) strain was identical to that of the first episode) which was still susceptible to HRZE. High Resolution Computed Tomography (HR CT) showed large pulmonary cavities. Treatment with HRZE was started. There was no clinical improvement in the first 4 weeks. Considering the slow culture conversion in the first TB episode and the fact that it concerned a relapse case, TB drug plasma concentrations were measured. A rifampicin peak concentration of 5.6 mg/L at 2 hours post dose (C2) was found. The isoniazid peak plasma concentration was undetectable (< 0.025 mg/L) at C2 and the pyrazinamide peak plasma concentration was 8.3 mg/L. After dose adjustment of rifampicin from 600 to 1200 mg, its peak plasma concentration increased to 15.2 mg/L. Dose adjustments of isoniazid and pyrazinamide did not lead to peak plasma concentrations within the target ranges (0.04 mg/L and 12.2 mg/L, respectively). Nevertheless, within 2 weeks after increasing the drug doses, the patient's productive cough diminished and his appetite approved. Sputum culture conversion occurred 6 weeks after dose adjustments. The patient did not experience adverse events to the higher dosages of rifampicin, isoniazid and pyrazinamide. His clinical condition was excellent at completion of treatment in March 2010 and remained well during a follow-up period that lasted until October 2010.

Patient 2 – treatment failure in a patient with relapse TB and diabetes mellitus

A 59-year-old Indian man with a history of type II DM and alcohol abuse was diagnosed with pulmonary TB in India in November 2006. He was treated until April 2007 and said to have been well adherent to treatment. Within 8 months after completing treatment, he was diagnosed with active TB in The Netherlands. The HR CT of the chest showed bilateral cavernous and endobronchial lesions. Standard treatment with HRZE under DOT was started. The MTB strain was susceptible to HRZE. One month after start of treatment,

pyrazinamide was stopped because of gastro-intestinal intolerance. There were no signs of hepatotoxicity. Sputum remained culture positive for more than 4 months (indicating treatment failure according to the Dutch NVALT guidelines).[16] Drug susceptibility testing showed that the MTB strain was still susceptible to all first-line TB drugs. Rifampicin plasma concentrations were determined and revealed a peak concentration of 4.1 mg/L at 2 h post dose. Dose adjustment took place (from 600 to 1200 mg), upon which the C2 plasma concentration increased to 22.4 mg/L, reflecting the nonlinear pharmacokinetics of rifampicin.[15] Sputum culture conversion occurred 1 month later. The patient gained weight and the cough disappeared. Despite gastrointestinal problems at the onset of treatment, the patient completed treatment without any further adverse events. The total treatment duration was 11 months. After more than 1.5 years of follow-up, there were no signs of relapse.

Patient 3 – second relapse TB after gastric surgery in the past

A 66-year-old Caucasian man with a history of Billroth II gastrectomy (1980), ischemic heart failure and chronic obstructive pulmonary disease (COPD, gold III), was successfully treated with self-administered 2HRZE/4HR for pulmonary TB in 2004. In December 2007, he developed relapse TB caused by an identical, drug susceptible MTB strain. He was treated with 2HRZE/4HR again (unsupervised). In December 2008, the patient returned with a second relapse of pulmonary TB. He presented with weight loss of 10 kg (BMI 13.7 kg/m^2). This second relapse was confirmed by identical MTB genotypic fingerprints to those of the previous episodes. Treatment with HRZE was started and the patient was admitted to our centre. Plasma concentrations of rifampicin and pyrazinamide were obtained considering the possibility of drug absorption problems in this patient as a consequence of gastric surgery in the past. Peak concentrations were low (4.0 mg/L for rifampicin and 10 mg/L for pyrazinamide) and occurred delayed (at 4 h post dose) for rifampicin. Because of the low BMI of the patient, protein-unbound (free) concentrations of rifampicin and pyrazinamide were measured as well, since a change in the ratio of unbound versus total (bound plus unbound) concentrations (i.e. the free fraction) may affect the interpretation of total concentrations.[18] However, both unbound fractions and plasma albumin levels were normal. Therefore, the doses of isoniazid, rifampicin and pyrazinamide were adjusted (H from 200 to 250 mg, R from 450 to 600 mg, Z from 1250 to 2000 mg). The pyrazinamide peak plasma concentration reached the target range (43 mg/L) with this dose adjustment, and the rifampicin peak plasma concentration did so after another dose adjustment (to 900 mg; C6: 8.5 mg/L). Sputum culture conversion occurred within 8 weeks after the second dose adjustment of rifampicin. The higher doses were tolerated well. The patient recovered with treatment under DOT in July 2009. Unfortunately, he died of progressive heart failure a few months later.

Patient 4 – relapse TB in a diabetes patient with extensive cavitary disease

A 42-year-old Caucasian man with a history of type II DM and schizophrenia completed treatment with 2HRZE/4HR under DOT for susceptible pulmonary TB in July 2007. In April 2008, he presented with relapse pulmonary TB that was susceptible to HRZE. The HR CT showed bilateral multiple small consolidations, large cavities in the left upper lobe and partial collapse of the right lung, together with traction bronchiectasis. Treatment with HRZE was started again. Sputum culture conversion occurred within 6 weeks after start of

treatment, but clinical and radiologic response were slow. Rifampicin plasma concentrations were measured after 12 weeks of treatment and the peak plasma concentration was 2.3 mg/L at 2 h post dose. Dose adjustment of rifampicin from 600 to 900 mg took place and resulted in an improved peak plasma concentration of 8.2 mg/L. The C4 en C6 plasma concentrations however, were 3.6 and 1.6 mg/L respectively, and it was decided to increase the dose to 1200 mg in order to achieve higher exposure. Clinically, the patient improved from the time of the final dose adjustment. A control CT scan 3 months later showed slight improvement, but the large cavities in the left upper lobe were unchanged. Positron Emission Tomography (PET) showed little fluorodexoglucose uptake in the lesions and therefore, we decided to refrain from surgery. Supervised treatment was continued for 9 months. No adverse events occurred. After more than 1.5 years of follow-up, the patient did not show signs of relapse TB.

Discussion

In our setting, TDM proved to be of help in detecting low TB drug plasma concentrations as one of the possible causes of slow treatment response, treatment failure or relapse TB, and in monitoring the effects of step-wise increases in drug doses. We presented four patients with relapse TB, all of whom appeared to have peak plasma concentrations of TB drugs below the target range. In most cases, increased drug doses resulted in peak plasma concentrations within the target range and clinical response to treatment, while no adverse events occurred.

Generally, the applicability of TDM depends on the fulfilment of a number of criteria.[13,15] First, there should be a large inter individual variation in drug plasma concentrations, which is the case in TB treatment.[10]

Second, there should be a clear relationship between drug plasma concentrations and response (which can either be therapeutic or undesirable, toxic response). The association between TB drug plasma concentrations and treatment response is incompletely understood. A limited number of *in vitro* and murine studies have shown that the ratio between the area under the plasma concentration curve (AUC) and the minimum inhibitory concentration (MIC) is the pharmacodynamic index that best explains the effects of isoniazid, rifampicin, pyrazinamide and ethambutol.[19-24]

Findings from a number of studies in humans pointed towards an association between low TB drug concentrations and poor treatment outcome,[3,4,25] but this association has been questioned by findings from other studies.[12,26]

As a third criterion for TDM, the drug concentration range in which a drug is effective and tolerated well, should be known and narrow. As mentioned previously, the target ranges that are currently used represent normal concentrations that can be expected after standard doses of TB drugs. As the standard doses are generally effective and tolerable, these concentrations seem suitable as target ranges.[10]

The fourth criterion for the applicability of TDM is that there should be no alternative, easier measurable indicator of treatment response available. Although clinical response and sputum smear or culture conversion are markers of TB treatment response,[27] they are typically assessed after weeks of treatment, when the emergence of resistance or treatment failure may already have occurred.[2]

Obviously, TB treatment does not fulfill all requirements for TDM, mainly because of gaps in knowledge and understanding of the pharmacokinetics and –dynamics of TB drugs. Sharing clinical experience of using TDM to individualize TB treatment could help to fill parts of those gaps.
We have presented a series of four cases only. The results are therefore not generalizable. Nevertheless, we think that this case-series illustrates the value of TDM as a tool to assess whether inadequate response to TB treatment can be attributed to suboptimal exposure to TB drugs in patients with drug-susceptible TB. More research is needed to evaluate the TB drug plasma concentration – response relationship as a basis for TDM in TB treatment.

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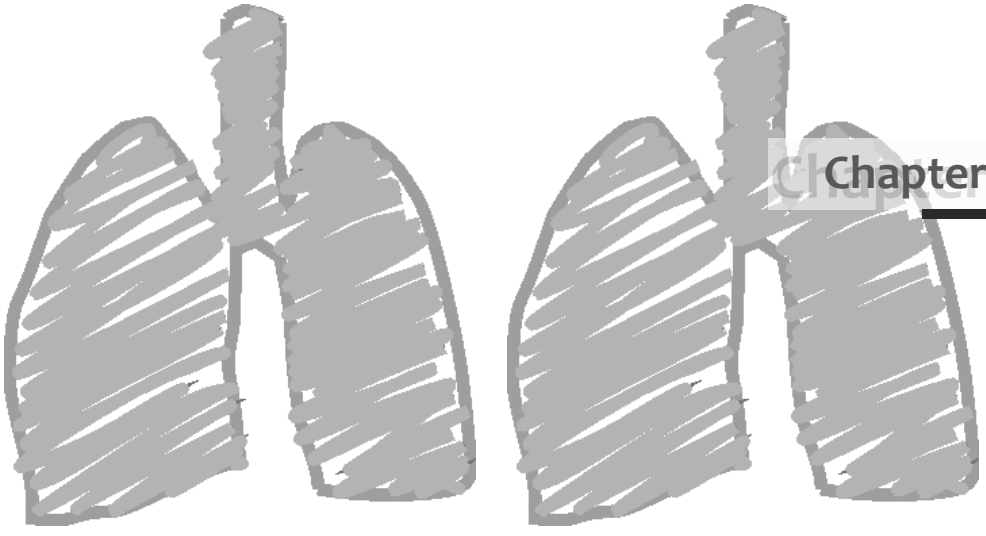
Pharmacokinetic studies in patients with Non-Tuberculous Mycobacterial lung infections



Chapter 8

Abstract

Concentrations of antimycobacterial drugs are an intermediary link between doses administered and eventual response to the drugs. Few pharmacokinetic studies have focussed on drug treatment for non-tuberculous mycobacterial (NTM) disease, although favourable treatment response occurs in just over 50% of patients despite drug treatment for at least one year. **Purpose of the study**: a prospective, descriptive pharmacokinetic study was performed to assess the plasma pharmacokinetics of rifampicin, ethambutol, clarithromycin, 14-OH-clarithromycin, azithromycin, isoniazid and moxifloxacin. Intensive pharmacokinetic sampling was performed in 14 patients with clinically relevant NTM lung disease. Pharmacokinetic parameters were assessed and compared with available data from the literature. **Results**: exposure to clarithromycin when combined with rifampicin was very low (geometric mean $AUC_{0\text{-}12h}$: 2.6, range 1.6-3.2 h*mg/L , C_{max}: 0.3, range 0.1-0.7 mg/L). The mean parent-to-metabolite ratios were 0.4 and 0.3 for $AUC_{0\text{-}12h}$ and C_{max}, instead of the typical ratio of around 3, probably reflecting increased metabolism of clarithromycin to its (virtually inactive) 14-OH-metabolite. Exposure to rifampicin was relatively high, with all patients having a rifampicin C_{max} value within the reference range. The majority of ethambutol C_{max} values were within the reference range. **Major conclusion**: the current study re-emphasizes the relevant pharmacokinetic interaction between clarithromycin and rifampicin. This calls for a re-evaluation of the dosing strategies in NTM lung disease, as suboptimal drug exposure may contribute to inadequate response to NTM treatment.

1. Introduction

The incidence of Non-tuberculous mycobacterial (NTM) lung infections is increasing worldwide due to various factors including improved detection methods, greater awareness of clinicians, advancing age, increasing incidence of COPD, the widespread use of immunomodulating drugs and possibly an increasing environmental exposure to these bacteria.[1,2] In The Netherlands, the incidence of NTM lung disease has also increased in the past years.[3] The Dutch NTM lung disease patient population differs from that in other countries in terms of a predominance of male sex (>60% men), cavitary disease (<10%), underlying lung diseases (>75%) and the causative NTM species, among which *M. avium* is predominant.[3-4] Successful treatment for NTM infections is hampered by misinterpretation of drug susceptibility patterns of the causative mycobacteria, co-morbidities including underlying lung disease[5] and possibly non-adherence to drug treatment due to adverse effects.[2]
Unfortunately, there is limited evidence for currently recommended drug treatment regimens; these regimens are mostly based on single centre, non-comparative studies and only few prospective clinical trials have been performed to compare possible treatment combinations.[1-2]

The relationship between the doses of the drugs applied and the drug concentrations achieved in patients (pharmacokinetics, PK) and the relationship between these concentrations and response (pharmacodynamics, PD) are important knowledge gaps in NTM lung disease treatment.[1,2,6] In NTM lung disease, very few studies have been performed to study drug concentrations as an intermediary link between dose and effect.[7-11]
In contrast, the PK of drugs in tuberculosis (TB) treatment have been studied extensively in the past (reviewed in[12-13]) and it has become evident that suboptimal exposure to TB drugs can result in inadequate response to TB treatment.[14-16] In our setting, measurement and interpretation of TB drug concentrations (Therapeutic Drug Monitoring, TDM) proved helpful in detecting low TB drug plasma concentrations as one of the possible causes of slow treatment response, treatment failure or relapse TB, and in monitoring the effect of step-wise increases in drug doses.[16] Similarly, insight into the pharmacokinetics and pharmacodynamics of drugs in NTM infections is urgently required to better comprehend how adequate response is achieved and when drug resistance and drug-related toxicity emerge.

In this study, we have determined the plasma pharmacokinetics of rifampicin, ethambutol, clarithromycin and its metabolite hydroxy-clarithromycin (OH-clarithromycin, which is far less active against *M. avium complex* than the parent drug), azithromycin, isoniazid and moxifloxacin in a series of patients in The Netherlands with clinically relevant NTM lung disease and we have compared the results to other series from the literature.

Materials and Methods

1 Subjects

Adult patients with *M. avium complex, M. kansasii* or *M. malmoense* pulmonary infections as diagnosed according to the American Thoracic Society (ATS) guideline,[2] were eligible for the

study and were recruited from November 2010 until November 2011. Patients were either newly diagnosed or currently receiving treatment. We included patients on daily treatment with at least rifampicin and ethambutol and optionally with isoniazid, clarithromycin, azithromycin or moxifloxacin. Patients with hepatic or renal dysfunction, pregnancy, cystic fibrosis or HIV infection were excluded, as these conditions may affect PK. All patients provided written informed consent, the study was approved by the Ethical Review Board of University Medical Centre Nijmegen, The Netherlands and was registered under www.clinicaltrials.gov: NCT01051752.

2 Design and procedures
A prospective, descriptive pharmacokinetic study was performed, using the standard two-stage approach. With this approach individual pharmacokinetic parameters are estimated in the first stage. In the second stage, population characteristics of each parameter are derived by obtaining measures of central tendency and spread of the subjects' individual parameters. The patients were treated with approved drugs supplied by our institution for at least two weeks to achieve steady state before the pharmacokinetic sampling day. Adherence in the two weeks preceding the sampling day was monitored with a medication diary in which patients recorded the time of drug intake.

Patients refrained from the intake of food from 00.00 a.m. at the PK sampling day. At 09.00 h the patients ingested all prescribed drugs on an empty stomach, under supervision of study personnel. In the case of clarithromycin, which is dosed twice daily, the second dose of 500 mg was taken 12 h later under supervision of the nurse of the hospital ward. Patients were allowed to eat, drink and take other medications starting from 12.00 am. A full pharmacokinetic curve was recorded. Serial venous blood samples were collected just prior to, and at 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 12.0 and 24.0 h post dose, or until 12.0 h post dose for clarithromycin. Plasma was separated immediately, frozen to -80 °C and transported on dry ice for bioanalysis.

3 Bioanalysis
Total (protein bound plus unbound) plasma concentrations of rifampicin, ethambutol, clarithromycin and its metabolite 14-OH-clarithromycin, and moxifloxacin were assessed by validated high performance liquid chromatography (HPLC) methods as described before.[17-19] Isoniazid was measured with liquid-liquid extraction followed by ultra performance liquid chromatography (UPLC) with ultraviolet (UV) detection. Accuracy was between 97.8% and 106.7%, dependent on the concentration level. The intra- and interassay coefficients of variation were less than 13.4 % (dependent on the concentration) over the range of 0.05 -15.1 mg/L. The lower limit of quantitation for isoniazid was 0.05 mg/L. Isoniazid containing samples were stable (<5% loss) for at least 12 months at -80°C. Azithromycin was measured with LC-MS/MS. Accuracy was between 98.0 and 101.3%, dependent on the concentration level. The intra- and interassay coefficients of variation were less than 7.4% over the range of 0.1 mg/L to 5.0 mg/L. The lower limit of quantitation was 0.1 mg/L.

4 Pharmacokinetic analysis
Pharmacokinetic parameters were assessed with non-compartmental methods using WinNonLin version 5.0 (Pharsight Corp., Mountain View, California). The highest observed

plasma concentration was defined as C_{max}, with the corresponding time as T_{max}. If the concentration at 24 h post dose (C_{24}) was below the limit of quantitation, it was calculated using the formula $C_{24} = C_{last} * e^{(-\beta * (24-Tlast)}$, in which β is the first order elimination rate constant and T_{last} is the time of the last measurable concentration (C_{last}). β was obtained by least squares linear regression analysis on log C versus time, with the absolute slope of the regression line being β/2.303. The area under the time-concentration curve (AUC_{0-24}, or AUC_{0-12} for clarithromycin and OH-clarithromycin) was calculated using the log-linear trapezoidal rule from zero up to T=24 u or T=12 h. The apparent clearance of the drug (Cl/F) was calculated by dose/AUC_{0-24} or dose/AUC_{0-12} for clarithromycin. The volume of distribution was calculated by the equation Cl/F/ [β].

5 Statistics
Pharmacokinetic parameters were described by geometric means and range, apart from T_{max} which was presented as median and range. Based on previously published reference ranges or values, C_{max} values were dichotomized as either normal or low. Low concentrations were defined as <8 mg/L for rifampin,[20] <2 mg/L for ethambutol,[20] <2.5 mg/L for clarithromycin,[21] <0.3 mg/L for azithromycin[22] and <3 mg/L for isoniazid[20] based on standard weight-adjusted dosing. The median peak plasma concentration for moxifloxacin at a daily dose of 400 mg as assessed in TB patients in The Netherlands was 2.5 mg/L.[23]

Where possible in view of patient numbers, the effects of gender, age, body weight and BMI on the PK parameters were assessed using the independent sample t-tests on the log-transformed parameters (or the Wilcoxon rank sum test for T_{max}; comparison of subgroups e.g. men vs women) or Spearman's rho on untransformed parameters (correlation of parameters). All statistical evaluations were performed with SPSS for Windows version 20 (SPSS Inc., Chicago). P values of less than 0.05 were considered significant in all analyses.

Results

1 Subjects
Fourteen patients were included in the study. Their baseline characteristics, drug regimens used and doses applied are shown in Table 1. All patients were Caucasians, 10 (71%) were males. Four patients had disease caused by *M. avium*, three *M. intracellulare*, one *M. chimaera*, three *M. malmoense* and three by *M. kansasii*. Two patients had a relapse of *M. avium* infection, all others had their first episode of NTM lung disease. Disease caused by *M. avium, M. intracellulare* and *M. chimaera* was treated with rifampicin and ethambutol in combination with claritromycin or azitromycin, if the causative bacteria proved susceptible, in vitro, to clarithromycin. *M. malmoense* was treated with rifampicin and ethambutol (n=2), combined with moxifloxacin in one case. *M. kansasii* was treated with isoniazid, rifampicin and ethambutol. In one patient with isoniazid resistant *M. kansasii*, clarithromycin was used. The two patients with recurrent *M. avium* infection had treatment regimens that differed from the regimen recommended by the ATS. One had rifampicin combined with clarithromycin because of toxicity of ethambutol. The other did not use clarithromycin because of macrolide resistance in vitro.

Table 1. Characteristics of 14 patients with NTM infection and their drug regimens

Characteristics	
Male, n (%)	10 (71%)
Age (yrs)(median, range)	67.5 (43-85)
Weight (kg)(median, range)	70 (60-83)
BMI (median, range)	23.5 (19-30)
Used drug regimens	
RIF, E, CLR	5
RIF, E	2
RIF, E, AZM	2
RIF, E, H	2
RIF, E, MXF	1
RIF, E, Cfz, AMK, Cs	1
RIF, CLR	1
Drug dosages (mg/kg/dose, mean±SD)	
RIF (n=14)	8.5 ± 0.7
E (n=13)	16.0 ± 1.9
CLR (n=6)	7.1 ±0.8
AZM (n=2)	5.5
H (n=2)	3.9
MXF (n=1)	6.2

Abbreviations: RIF: rifampicin, E: ethambutol, CLR : clarithromycin, AZM: azithromycin, H: isoniazid, MXF: moxifloxacin, Cfz: clofazimine, AMK: amikacin, Cs: cycloserin

The clarithromycin dose used was 500 mg BID. Azithromycin dose was 250 mg once daily in one case and 500 mg once daily in the other. Adherence in the two weeks preceding the PK assessment was 100% according to the medication diaries. All but one patient had a full pharmacokinetic curve recorded. This patient had samples taken at 2, 4 and 6 hours post dose.

2 Pharmacokinetic analysis

In one out of six patients on clarithromycin, concentrations of clarithromycin and 14-OH-clarithromycin could not be analyzed as the volume of plasma remaining after analysis of rifampicin was not sufficient to analyze other drugs. In another patient on clarithromycin, only four subsequent concentrations in the PK curve were measurable at the limit of quantitation, which shows a very low exposure to clarithromycin but did not allow the calculation of an $AUC_{0\text{-}12}$.

The resulting PK parameters of rifampicin, ethambutol, clarithromycin, 14-OH-clarithromycin and azithromycin are summarized in Table 2. Figure 1 and 2 display the average PK curves of rifampicin, ethambutol, clarithromycin and 14-OH-clarithromycin. As to rifampicin, a roughly three fold interindividual variability in AUC_{0-24} and C_{max} was observed. All patients reached a C_{max} value within the reference range of rifampicin (>8 mg/L). With respect to ethambutol, one out of 13 patients had an ethambutol C_{max} below the reference range (2 mg/L). C_{max} values of clarithromycin were far below the reference value of 2.5 mg/L in all 5 patients in whom clarithromycin could be measured (geometric mean 0.3 mg/L, range 0.1-0.7).
The parent drug to metabolite ratio for clarithromycin to 14-OH-clarithromycin has been described as 3:1, but the mean value was 0.39 (C_{max}) and 0.33 (AUC_{0-12h}) in our patients. In both patients who used azithromycin, the C_{max} reference value (>0.3 mg/L) was not reached. The two patients on isoniazid had AUC_{0-24} values of 8.4 and 11.1 h*mg/L and C_{max} values were 5.0 and 4.9 h* mg/L, i.e. within the reference range. The AUC_{0-24} and C_{max} for moxifloxacin in one patient were 20.5 h*mg/L and 2.2 mg/L respectively.
We did not find clear correlations between gender, age, body weight or BMI on the one hand and AUC or C_{max} of rifampicin, ethambutol and clarithromycin or 14-OH-clarithromycin on the other hand, but obviously the numbers of patients were small.
The AUC_{0-12} ofclarithromycin and 14-OH-clarithromycin did not correlate with the AUC_{0-24} of rifampicin (r=0.1, p=0.01 and r=0.1, p=0.01 respectively).

Table 2. Pharmacokinetic parameters of rifampicin, ethambutol, clarithromycin and azithromycin in patients with NTM disease [a]

Pharmacokinetic parameter	RIF N=14	E N = 13	CLR N=5	14-OH-CLR N=5	AZM N=2
AUC_{0-24} (RIF, E, AZM) or AUC_{0-12} (CLR, 14-OH-CLR) (h.mg/L)	45.9 27.8-80.6	24.2 12.6-46.7	2.6 [b] 1.6-3.2	6.6 2.0-12.8	1.9 1.4-2.6
Cmax (mg/L)	12.3 8.6-24.9	3.1 1.5-5.3	0.3 0.1-0.7	1.0 0.4-1.9	0.18 0.17-0.2
Tmax (h)	2.0 1.0-4.0	3.0 1.5-6.0	2.0 2-12	3.0 3-6	5.25 2.5-8.0
Cl/F (L/h)	13.1 7.4-21.6	46.5 25.7-79.3	188.9 [b] 154.4-308.2		182.0 175.0-189.3
Vd/F (L)	30.4 16.5-45.2	671.6 338.6-1356.4	1207.4 [c] 602.2-4817.1		2979.6 1913.1-4640.8
t½(h)	1.6 1.2-2.7	10.0 6.6-16.2	4.1 [c] 2.4-10.8	4.0 2.6-7.2	11.3 7.6-17.0

a. Geometric means and range, apart from median and range for Tmax. Abbreviations of pharmacokinetic parameters: see the text.
b. Based on data for n=4 patients
c. Based on data for n=3 patients
d. Abbreviations: RIF: rifampicin, E: ethambutol, CLR : clarithromycin, 14-OH-CLR: 14-OH-clarithromycin, AZM: azithromycin

Figure 1. Pharmacokinetics of rifampicin (n=14) and ethambutol (n=13), mean and standard deviations

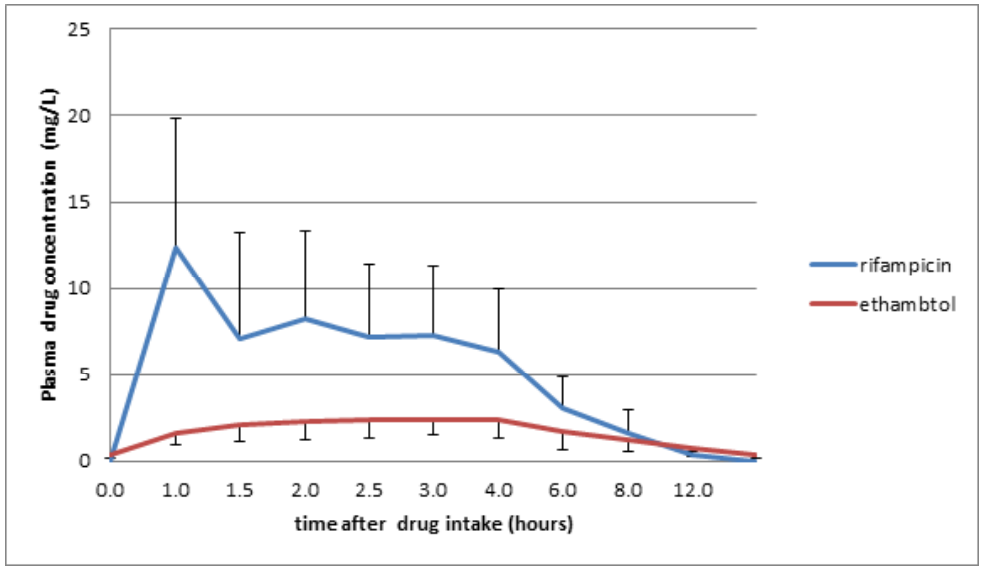
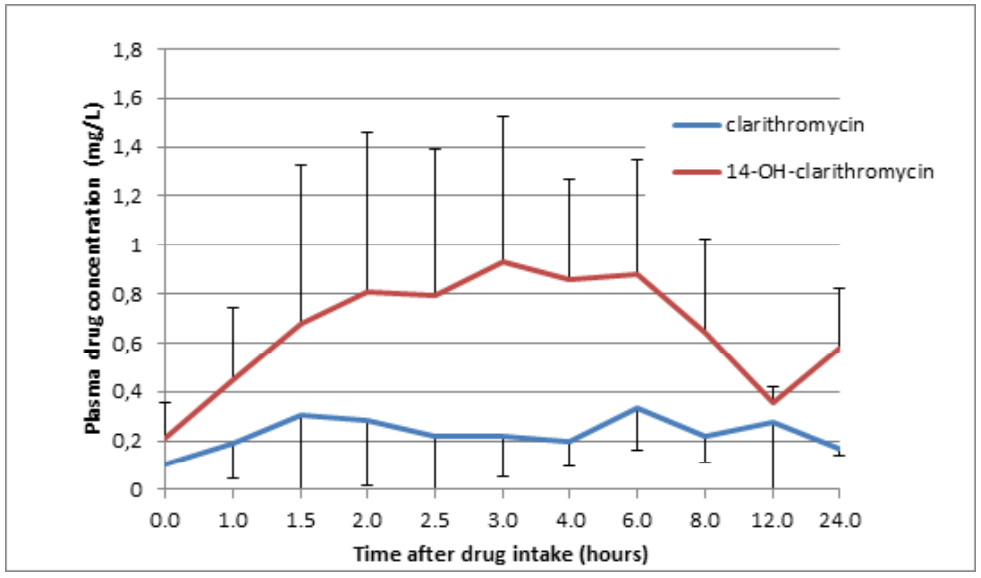


Figure 2. Pharmacokinetics of clarithromycin and its active metabolite 14-OH-clarithromycin (n = 5), mean and standard deviations



4 Discussion

This study evaluated the steady-state pharmacokinetics of antimycobacterial drugs, obtained by intensive pharmacokinetic sampling, in a series of NTM lung disease patients who were typical to the Dutch NTM patient population in terms of race, male sex, age and a relatively low body weight.[3]

The most important finding from this study is that exposure to clarithromycin is low when combined with rifampicin. All 5 patients in whom clarithromycin was measured had C_{max} values far below the reference value of 2.5 mg/L for clarithromycin.[21] In addition, the average AUC_{0-12} for clarithromycin after a dose of 500 mg at steady-state (geometric mean: 2.6 h* mg/L) was much lower than that described without co-administration of rifampicin (circa 20 h*mg/L).[21]

The mean parent to metabolite ratio for the C_{max} and AUC for clarithromycin were 0.3 and 0.4, respectively, which is much lower than the typical ratio of 3:1.[7,21] This reflects an increased metabolism of clarithromycin to its main 14-OH-metabolite. The latter is 10-30 times less active against *M. avium complex*, in vitro, than the parent drug.[24] These findings are in agreement with available data from the literature, summarized in table 3,[7-10] previously demonstrating low C_{max} and AUC levels of clarithromycin in the presence of rifampicin. Most probably, these effects are the result of the induction by rifampicin of the metabolic enzyme that catalyzes the hydrolysis of clarithromycin.[8]

Table 3. Pharmacokinetic studies in NTM disease studying co-administration of clarithromycin and rifampicin [a,b]

Author (number of sampling points)	Drugs (number of patients)	Average Dose	Average AUC (h.mg/L)[c]	Average, C_{max}(mg/L)
Wallace (1995) (1)	RIF (12)	600 mg QD	NA	NA
	CLR (12)	500 mg BID		0.7
	14-OH CLR (12)			1.8-1.9
Peloquin (1996)[d] (2)	RIF (12)	NA	NA	10.3
	CLR (21)	10.6 mg/kg QD		0.58
	14-OH CLR (21)			1.75
Alffenaar (2010) (full PK curve)	RIF (8)	8.8 mg/kg QD	25.8	4.9
	CLR (8)	7.5 mg/kg QD	2.9	1.0
	14-OH CLR (8)		8.6	1.5
Van Ingen (2012) (2)	RIF (326)	9.6 mg/kg QD	68.4	18.8
	CLR (39)	14.3 mg/kg daily	5.3	1.25
	14-OH CLR (39)		NA	NA
This study (2012) (full PK curve)	RIF (14)	8.5 mg/kg QD	45.9	12.3
	CLR (5)	7.1 mg/kg BID	2.6	0.3
	14-OH CLR (5)		6.6	1.0

a. Abbreviations: n: number of patients studied, NA: not available, RIF: rifampicin, CLR: clarithromycin, 14-OH-CLR: 14-OH-clarithromycin
b. Measures for central tendency (e.g. mean, median, geometric mean) differ between studies
c. AUC data differ from study to study: Alffenaar et al assessed AUC_{0-24} for RIF, CLR and 14-OH-CLR; van Ingen et al assessed $AUC_{0-6/7}$ for RIF and CLR; and the current study assessed AUC_{0-24} for RIF and AUC_{0-12} for CLR and 14-OH-CLR
d. Data are shown for the 'NJC population' described in this study, not for the 'referral patients'

The low clarithromycin plasma concentrations demonstrated in this study are likely to be very relevant for treatment outcome. Macrolides exert so called time-dependent inhibition, which requires that the drug concentrations should be above the MIC value of the bacteria for a certain minimal amount of time (T>MIC).[21]
The low clarithromycin AUC_{0-12} and C_{max} values upon co-administration of rifampicin will inevitably result in a decrease in T>MIC in plasma. For mycobacteria it is not yet clear, though, how long this amount of time should be. Furthermore it should be underlined that macrolides achieve significantly higher drug concentrations in the epithelial lining fluid (ELF) and alveolar macrophages, the potential sites of action of extracellular and intracellular replicating respiratory pathogens, respectively.[21] There are no actual data to show that the macrolide accumulation in tissues in humans is affected by the interaction with rifampicin. In a recent study in foals however, the concentrations of clarithromycin in ELF and in alveolar macrophages were lowered drastically after co-administration with rifampicin.[25]
Clearly, the interaction of clarithomycin with rifampicin may be one of the reasons for suboptimal response to drug treatment in NTM lung disease. Consequently there is a need for follow-up studies that evaluate the pharmacokinetics, safety/tolerability and efficacy of a higher dose of clarithromycin when combined with rifampicin. Alternatively, rifabutin may be used instead of rifampicin, as co-administration of this rifamycin has less effect on clarithromycin concentrations.[7] However, it should be noted that rifabutin and clarithromycin show a complex bi-directional interaction which may result in rifabutin-induced toxicity, including uveitis and polyarthritis.[21]
Azithromycin could also be used as an alternative to clarithromycin, as it is generally less prone to interactions and can be dosed once-daily. Our data on azithromycin relate to only two patients, which precludes firm conclusions. In the absence of follow-up studies at this moment, TDM may be used to optimize the exposure to all drugs in individual patients.[8,16]

Considering rifampicin, our data showed that all patients reached a rifampicin C_{max} value within the reference range (i.e. > 8mg/L). This may suggest that patients with NTM disease reach higher peak plasma concentrations of this pivotal antimycobacterial drug than patients with TB. For example, in a study among TB patients in Dutch TB clinics, using a similar intensive sampling scheme and the same analytical methods, we observed that 30% of the patients had C_{max} values below 8 mg/L (Magis-Escurra, et al. submitted). Similarly, an intensive PK sampling study of ours in Indonesian TB patients showed 21% of patients with rifampicin C_{max} values below 8 mg/L.[17]
Using other analytical methods and limited sampling, Van Ingen et al. also found only 5% of patients with rifampicin C_{max} concentrations below 8 mg/L among Northern American patients with NTM (*M.avium*) lung disease.[10] The higher C_{max} of rifampicin among patients with NTM disease versus TB patients may be due to faster absorption or smaller volumes of distribution. Furthermore we can not exclude that co-administration of isoniazid in all patients with TB (as compared to few patients with NTM lung disease) may somehow affect the pharmacokinetics of rifampicin. As to the *total* exposure of rifampicin, the current study with intensive sampling allowed for an accurate estimation of AUC_{0-24} of rifampicin (geometric mean: 45.9 h*mg/L) and this appeared to be only slightly higher than the geometric mean AUC_{0-24} in Dutch TB patients (41.1 h*mg/L, Magis-Escurra et al., submitted). Of note, most NTM are known to be much less susceptible (i.e. having much higher minimum inhibitory concentrations [MIC]) to rifampicin than *M. tuberculosis*[26] but

there are no clinical data showing what C_{max}/MIC or AUC/MIC values should be reached for this exposure-dependent drug in NTM disease or TB. As to ethambutol, only one patient had a low C_{max} (1.4 mg/mL) and AUC_{0-24} (12.6 h.mg/L) of ethambutol. Similarly, ethambutol C_{max} reference values are often reached in TB patients.[17] In contrast, Van Ingen et al. found that 48% of patients had ethambutol C_{max} values below the reference values (2-6 mg/L) in a Northern American population of patients with NTM lung disease.[10]

The strength of this study compared to the few other pharmacokinetic studies in NTM disease was the intensive sampling of patients, resulting in accurate estimates of pharmacokinetic parameters in all individuals. However, the study was limited by the small sample size which restricted statistical power to detect possible pharmacokinetic differences between patient subgroups.

In summary, this study confirmed and re-emphasized that exposure to clarithromycin is very low when rifampicin is co-administered in patients with NTM disease. In addition, results from this study suggest that peak plasma concentrations of rifampicin are higher in patients with NTM disease compared to patients with TB. There remains an urgent need to re-evaluate dosing strategies in NTM lung disease, to study the pharmacokinetics, safety/tolerability and efficacy of higher doses of clarithromycin when combined with rifampicin, and to evaluate azithromycin and rifabutin as alternative macrolides and rifamycins in combatting NTM.

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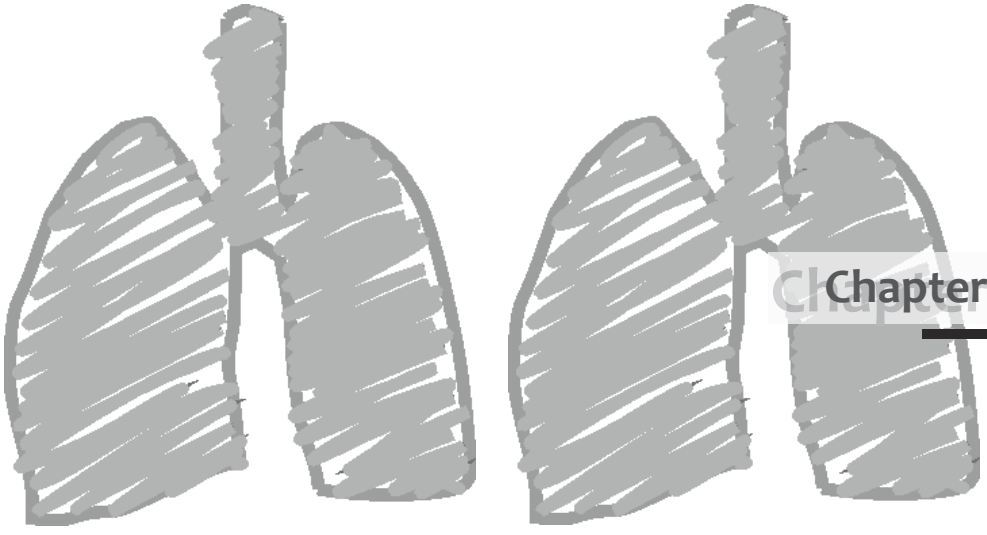
Background

Tuberculosis (TB) has ravaged humans since ancient times. Signs of the disease could already be observed in pharao's tombs, where mummies showed signs of "Pott's spine disease" leaving mutilating and disabling kyphoscoliosis. Although we dispose of effective treatment for TB since the sixties of the last century, not a single country in the world has yet managed to eradicate this disease.
TB is caused by bacteria from the Mycobacterium tuberculosis complex and consists of several subtypes. Although the global incidence and mortality is declining still a 9 million new TB patients are registered annually with 1.4 million annual deaths. Incidence rates vary enormously from 5/100.000 in Scandinavian, some European countries and the United States of America to well over 300/100.000 in South Africa.
Non tuberculous mycobacteria (NTM) are becoming frequently recognized as real pathogens. They are culprits of a disease resembling TB with the difference of being a non-communicable and showing a rising incidence especially in countries where TB incidence is declining. This increase is caused by various factors including improved detection methods, greater awareness of clinicians, advancing age, increasing incidence of COPD, the widespread use of immune modulating drugs and possibly an increasing environmental exposure to these bacteria.
This thesis gives an overview of epidemiological, diagnostic and pharmacological research from Dekkerswald, a Dutch tertiary referral centre for patients with TB and NTM infections. All studies in this thesis deal with mycobacterial infections, mostly tuberculosis but also one chapter on NTM infections.

Summary and general discussion



Chapter 9

Summary of the chapters

Introduction
In the introduction of this thesis ***(chapter 1)*** a historical perspective, pathogenesis, diagnosis and treatment of TB and NTM are described**.** Subsequently we zoom in to pharmacokinetics of TB treatment helping to understand that TB treatment harbours many possible threats for treatment failure, relapse and acquired drug resistance. Finally, an overview of Dutch TB control is given, explaining the function of clinical TB consultants and the TB referral centers.

Epidemiological studies
The thesis starts with a descriptive study ***(Chapter 2)*** of TB patients in our centre, admitted during the period 2000-2005, and provides an insight in the demographic, clinical and social data of this population. A total of 166 patients with 37 different nationalities were analyzed. Tertiary referrals accounted for 98% of all hospitalizations. Most patients (68%) were referred for clinical reasons and 32% were referred for social reasons such as homelessness, substance misuse and psychiatric diseases.
Severe hepatotoxicity was the principal clinical reason for referral, followed by delayed response to treatment, need of surgical interventions, combined HIV-TB infection, drug resistance problems, and need for isolation. Of the drug resistant strains, 94% were isolated from patients of foreign origin. Toxicity and side effects of treatment often led to changes in treatment (40%). Ten percent of patients were seropositive for HIV and many of them (82%) experienced adverse effects, mainly hepatotoxicity. Extrapulmonary TB was diagnosed significantly more often in the foreign patient group.
The median admission period for the total population was 10.1 weeks, showing no differences between patients admitted for clinical or social reasons or country of origin. Eighty-five percent of the population completed treatment, six percent was lost to follow up and TB mortality was five percent.
In conclusion, the TB population treated in Dekkerswald had excellent treatment success rates despite the complexity and diversity of this group. Regarding the increased number of admissions to our centre since 2005, we seem to be meeting a considerable demand. When TB incidence began to decline, sanatoria were no longer needed at large scale, but TB will never be eliminated completely and it is therefore important that some of these specialized institutions remain active to preserve and optimize expertise.

The subsequent chapter ***(chapter 3)*** describes the retrospective analysis of all *Mycobacterium bovis* TB in The Netherlands during 1993-2007 after our attention was drawn by three Dutch patients with *M. bovis* disease in our Centre.
We analyzed data from 231 patients for clinical, demographic, treatment, outcome characteristics and for risk factors. Disease was mainly extrapulmonary (n=136; 59%). Although 95 patients had pulmonary disease, person-to-person transmission did not occur, as shown by structural DNA fingerprinting analysis.
Lymph node TB was more likely to develop in women ($p<0.0001$), whereas pulmonary *M. bovis* disease developed more frequently in men ($p<0.0001$). Diagnosis was accurate but the determination delay led to inadequate treatment in 17% of the cases. The proportion of deaths from *M. bovis (5%)* was higher than that for *M. tuberculosis* disease (2%). From this

study it was concluded that the prevalence of *M. bovis* disease in The Netherlands (1.4%) is comparable to that in other countries in which control programs for *M. bovis* infection are enforced. Gender differences in clinical features and mortality rates were found in our cohort of patients. The disease now mainly affects elderly native Dutch citizens (60%) and immigrants from Morocco (23%). Anti-TNF-α treatment is an emerging cause of endogenous reactivation and *M. bovis* TB may reactivate slower than *M. tuberculosis* TB.

Diagnostic studies

In the following chapter ***(chapter 4)*** we assessed the accuracy of registration of culture results in the TB register and studied the characteristics of patients notified with non-culture confirmed TB and the quality of TB diagnosis. Patient records of patients notified with non-culture confirmed TB in the period 2007-2009 were reviewed at the Public Municipal Health Services (PMHS). These data were compared to the information in the NTR. Cases were subsequently classified according to the TB case definition of the European Centre for Disease prevention and Control (ECDC) as; '*no', 'possible', 'probable'* or '*confirmed'* TB. Laboratory confirmation data of 6% of notified TB cases in the NTR were incorrect or incomplete and the overall percentage of culture confirmed cases was corrected from 70% to 71%. In total 63% of non-culture confirmed cases were ETB. A total of 42% of non-culture confirmed TB diagnoses were established with other laboratory evidence such as positive Polymerase Chain Reaction or positive Ziehl Neelsen staining, or histology suggestive of TB. In total 164 out of 516 (32%) of the non-culture confirmed cases represented early diagnosis of extra pulmonary TB due to active case finding by physicians of PMHS, and no culture was attempted. In clinical extra pulmonary cases culture was not done because biopsy material was not suitable for culture anymore. 56% of non-culture confirmed cases was classified (according to the ECDC case definition) as '*possible TB*', 35% as '*probable TB*', and 6.8% as '*confirmed TB*'. Thirteen cases could not be classified due to missing data. Most 'possible' TB cases were those with a high likelihood of infection, diagnosed through active case finding by physicians of the PMHS. 'Probable' TB cases were mostly diagnosed in the hospital, culture was attempted in 60% of the cases but remained negative. In 31% of the cases the body material was retrieved, but delivered in the wrong condition to be cultured. Hospital clinicians should become aware of the importance of their contribution to registration and surveillance to control TB. On the other hand, advanced training of public municipal health staff may further improve quality of the NTR.

Another study concerning diagnostics ***(Chapter 5)*** provides insight in the patient- and clinical characteristics of patients with spinal TB and the course of diagnostic delays of spinal TB from 2000-2011 in The Netherlands. We performed a descriptive, retrospective study. Data from the Netherlands Tuberculosis Registry (NTR) were studied, completed with basic demographic data and data considering patients-, doctors- and total diagnostic delay retrieved from the patient records at the public municipal health services.
A total of 274 cases were studied at the PMHS. We found a median diagnostic delay of five months and this remained stable during 2000-2011. Sex and age groups were associated with significant differences in diagnostic delay (resp. male 4.5 vs female 5.5 months, p=0.004 and 0-34 years 4.5 months, 35-64 years 5.75 months and > 65 years 5.0 months, p=0.006). No difference was observed between origin of patients, patients presenting with TB risk factors or with neurological symptoms. Typical TB symptoms (back pain, night

sweats and weight loss) at presentation surprisingly lead to significantly increased doctors' delay (typical symptoms 4.0 vs no typical symptoms 2.0 months, p=0.05).
We concluded that refresher courses should be offered continuously both to family physicians and clinical specialists in order to raise the awareness and knowledge of TB in the era of declining TB incidence in The Netherlands.

Pharmacokinetic studies
To improve outcome of TB treatment, a better understanding of anti-TB drug pharmacokinetics is indispensable. Information about drug exposure in fixed dosing regimens seems crucial now to understand mechanisms of treatment failure, relapse and emergence of drug resistance. In addition, rather than only applying fixed dosing regimens, physicians may have drug concentrations measured and may individualize the dose based on the measured concentration (Therapeutic Drug Monitoring, TDM). However, unawareness of the relevance of drug exposure for response, high patient burden, costs and time spent still keep clinicians from performing TDM on a large scale, both for current first line and new anti-TB drugs.
Variability in exposure to anti-TB drugs is one of the main factors related to treatment failure and the emergence of resistance. Therefore, total drug exposure, AUC_{0-24}, is the pharmacokinetic parameter probably most relevant to the efficacy of TB drugs. High patient burden, costs and time spent, still keep clinicians from performing Therapeutic Drug Monitoring (TDM) of current first-line drug regimens on a large scale.

In ***chapter 6*** we assessed population pharmacokinetic data especially concerning AUC_{0-24} for first-line TB drugs and moxifloxacin and developed limited sampling strategies. Intensive pharmacokinetic sampling was performed in 41 hospitalized patients from two TB referral centers in The Netherlands. Drug concentrations were assessed with validated HPLC methods and pharmacokinetic parameters were assessed with non-compartmental pharmacokinetic methods. Best subset selection multiple linear regression was performed to derive limited sampling equations predictive of the AUC_{0-24}. To assess the predictive performances of the models, residuals for each patient were calculated based on models fitted to a dataset where that patient was omitted.

Geometric mean AUC_{0-24} values for rifampicin, isoniazid, pyrazinamide, ethambutol and moxifloxacin were 41.1, 15.2, 380, 25.5 and 33.6 h*mg/L respectively. Limited sampling at two or three fixed sampling points allowed for an accurate and precise estimation of the AUC_{0-24} values of all drugs separately and simultaneously. Sampling at 1, 4 and 6 h best predicted the AUC_{0-24} of all drugs together.
In the absence of clinically validated target values for AUC_{0-24}, the derived average values for AUC_{0-24} from this population could be used as reference values in TDM. Limited sampling of AUC_{0-24} of TB drugs is feasible and allows for TDM at a larger scale in Europe.

In the next chapter ***(chapter 7)*** we provide insight in the current TDM practice in our centre. TDM is used in patients with drug-susceptible TB when abnormal TB drug concentrations are suspected (e.g. in case of HIV-infection, diabetes, gastro-intestinal abnormalities, malnutrition, or renal or hepatic dysfunction), when there is delayed sputum culture

conversion (i.e. after more than eight weeks of treatment), and in case of relapse TB. We present a brief clinical glossary of four relapse TB cases to illustrate the method of TDM and the value of individualized TB drug dosing with low TB drug concentrations.
In the four relapse cases increased doses of the first line TB drugs, especially rifampicin, resulted in adequate peak plasma concentrations and subsequently improved clinical response to treatment in these patients, while no adverse events occurred.

As stated before, the mainstay of the thesis remains TB disease, but NTM infections are an entity not to be neglected. Last but not least, we therefore shift to a pharmacokinetic study in NTM lung disease in the last chapter ***(chapter 8)***. Treatment outcomes for NTM infections are poor even if recommended regimens are applied. In NTM disease very few pharmacokinetic (PK) studies have been performed. We performed a descriptive PK study using 11 sampling points, to assess the plasma pharmacokinetics of rifampicin, ethambutol, clarithromycin, azithromycin, isoniazid and moxifloxacin and their active metabolites in a series of 14 patients with clinically relevant NTM lung disease. We compared our results with available data from the literature.

We learned that exposure to clarithromycin when combined with rifampicin was very low, and the mean parent-to-metabolite ratios were low as well. This probably reflects induction of metabolic enzymes by rifampicin. Exposure to rifampicin was relatively high, with all patients having a rifampicin C_{max} value within the reference range of over 8 mg/L. Similarly, all ethambutol C_{max} values were within the reference range between 2 and 6 mg/L. From this study we concluded that the relevant PK interaction between clarithromycin and rifampicin calls for a reconsideration of the dosing strategies in NTM lung disease, as suboptimal drug exposure may contribute to inadequate response to NTM treatment.

The table on the next page contains highlights of the thesis and their implications for future TB management and research.

Anthology of this thesis' findings and implications

	Query	Finding	Implication
Epidemiological studies	Chapter 2 **What characterizes patients admitted to a Dutch TB referral centre (2000-2005)?**	166 patients from 37 different nationalities, 68% hospitalized for medical reasons. Drug resistant TB occurs in 23%. Ten percent is HIV-positive. Good therapy response with interdisciplinary approach at our centre.	1. specialized centers meet an important demand. 2. TB expertise must be actively sustained. 3. consultants specialized in clinical TB care play a pivotal role in safeguarding expertise. 4. clinical TB expertise can be applied to improve TB care elsewhere in the EU/EEA region.
	Chapter 3 **Which was the result of screening for latent TB before starting TNF alfa blocking agents in two patients who developed M.bovis TB?** **Who is prone nowadays to develop bovine TB in The Netherlands?**	The two patients will receive TB prophylaxis with current guidelines. Prevalence bovine TB 1.4%, mainly older Dutch (59.7%) and Moroccan individuals (23.4%). Inadequate treatment in 17% of cases due to ignorance or slow determination and drug susceptibility testing.	1.the screen for latent TB is in need of a gold standard, evaluation of updated national guidelines and of a cardinal role for hospital TB coordinators. 2. faster determination and drug susceptibility for M.bovis; accessibility to advise treatment regimens.
Diagnostic studies	Chapter 4 **Was the accuracy of registration of culture results in the Netherlands TB Registry during 2007-2009 accurate? What characteristics had non-culture confirmed TB cases and which was their diagnostic accuracy?**	6% of notified TB cases in the NTR were incorrect. Culture confirmed cases was corrected from 70% to 71%. In total 63% of non-culture confirmed cases were ETB. 32% represented active case finding. 56% of cases was classified as 'possible TB', 35% as 'probable TB', and 6.8% as 'confirmed TB'.	1. awareness to culture biopsy and resection material should increase. 2. false positive diagnosis of TB disease in active case finding should be considered. 3. developing standard forms to complete NTR data. 4. advanced training to further improve quality of the registered data in the NTR.
	Chapter 5 **Did diagnostic delay in spinal TB worsen in the last decade?**	Diagnostic delays remained stable over the years. Classical TB presentation or likewise risk factors did not reduce degree of diagnostic delays.	1.TB expertise needs to be preserved, this applies even more so in extrapulmonary TB. 2.role of consultants for clinical TB is important to maintain this expertise.
Pharmacokinetic studies	Chapter 6 **Which were the population pharmacokinetics of first line anti-TB drugs and moxifloxacin from the two Dutch TB referral centers and can limited sampling strategies be developed to estimate AUC_{0-24} values of these drugs?**	AUC_{0-24} values for rifampicin, isoniazid, pyrazinamide, ethambutol and moxifloxacin were 41.1, 15.2, 380, 25.5 and 33.6 h*mg/L respectively. Limited sampling at two or three fixed sampling points allowed for an accurate and precise estimation of the AUC_{0-24} values of all drugs.	1. TDM with limited sampling and less invasive PK methods is needed to implement TDM as a standard procedure in European TB care.
	Chapter 7 **What happens in a TB referral center with patients suspected for low serum levels of anti TB drugs?**	TDM proved very useful in these selected cases with relapseTB. All cases showed lowered exposure to rifampicin. Higher dosage of rifampicin improved clinical status and rapidly led to culture conversion and termination of further relapses.	1.TDM should become widely available in European TB (referral) centers. 2.limited sampling and dried blood spot methods facilitate the use at larger scale of this valuable tool for TB treatment.
	Chapter 8 **What can we learn from pharmacokinetic data in patients with non-tuberculous mycobacterial lung infections?**	Exposure to clarithromycin with rifampicin was very low. Exposure to rifampicin was relatively high. The majority of ethambutol C_{max} values were within the reference range.	1. dosing strategies of in NTM lung disease need to be reconsidered to optimize treatment outcome. 2. evaluation of other treatment regimens is necessary

General discussion on future TB care and research

From this table the implications of our studies for future clinical TB care and prevention become evident. Tuberculosis care in an affluent country such as The Netherlands readily provides all required resources, yet tuberculosis is still the invincible disease it has been since ancient times. Referral centers and clinical TB consultants currently meet a considerable demand in The Netherlands and will remain needed at national level. Hospital TB coordinators will play an important role in the future. Their knowledge and expertise needs to be maintained as they will be the spider in the web of screening, prevention and quality monitoring in TB care. Data supply for TB surveillance registry will benefit from more actively involved local physicians. From an international and especially European perspective, the Dutch level of expertise may serve as an example in optimization of drug resistant TB treatment. Adapting more to active case finding policies can decrease TB incidence. On the other hand, our study results also nourish further TB research. They stress the need of gold standards in latent TB infections (LTBI). Equally important is the evaluation of national and European guidelines for screening and treatment of patients with LTBI. Clinical TB care would benefit from strategies accelerating *M.bovis* growth. Finally, therapeutic drug monitoring warrants simpler, less invasive and less time consuming methods. The considerable outcome variability in different laboratories need quality control programs to guarantee its quality in different centers. In the next paragraphs several of these implications are highlighted and discussed more extensively.

Hospital TB coordinators

In only five years time (2006-2011), the TB population of Dekkerswald has grown exponentially to a median population of 80 patients a year [unpublished data]. Migration has not increased anymore since 1994 and TB incidence rates have even shown a slight decline to a stable 1000 TB cases each year now. It appears the decreasing expertise of the health care professionals due to declining incidence rates indeed led to increased transfers to TB referral centers as was preluded in an article in 2002.[1,2]

In 2011 the Netherlands Scientific Board of Respiratory Disease and Tuberculosis Specialists (NVALT) adopted the idea of allocating a TB coordinator for each hospital. These coordinators will be responsible for all TB related matters in their hospital. By appointing TB coordinators, together with the official recognition by the NVALT of the four clinical TB consultants, we will probably be able to ensure the quality of TB diagnostics, treatment and hygiene in the hospitals in the coming era.

To consolidate this idea, regular updates for TB coordinators, as organized in 2011 and 2012 as a master course at the Erasmus Medical Centre, Rotterdam, should be sustained by the NVALT. Firstly, TB coordinators will acquire more knowledge of TB and will be trained in hospital tuberculosis hygiene measures **(*chapter 2 and 5*)**. Secondly, the role in latent TB infection (LTBI) screening before starting anti-TNF α blocking agents will be discussed **(*chapter 3*)**. Thirdly, updated treatment guidelines are to be discussed, if available. Finally their important role in data collection for the National Tuberculosis Registry (NTR), a means of surveillance, will be emphasized **(*chapter 4*)**. During the courses TB coordinators get familiar with the clinical TB consultants which will help to consult one of them in case of complex TB **(*chapter 2*)**. All these measures are necessary to preserve TB knowledge and experience of respiratory diseases specialists at a national level **(*chapter 2, 3, 4, 5*)**.

Drug resistant TB in the European Region

With the interdisciplinary approach to complex and drug resistant TB, Dekkerswald had excellent treatment outcomes.[3] Contrary to this, the overall treatment success in the European Union and European Economic Area (EU/EEA) is currently the lowest in the world (67%).[4] This is caused by less favorable treatment outcomes in some subregions of the EU/EEA which are responsible for 86% of all TB cases and 98% of MDR-TB cases of the EU/EEA.[5] A considerable number of these countries form part of the European Union, which allows its members free travel - to and from - within its borders.
Especially drug-resistant TB is now posing a real challenge to weak public health care systems of mainly former Soviet Union countries and TB is becoming a public health menace again in Europe. Problems of therapy errors, nonadherence, pharmacokinetic variability and most important nosocomial infections are leading continuously to acquiring drug resistance in these subregions. The fight against multi- and extensively drug resistant (MDR/XDR) TB in these EU/EEA subregions should become a priority effort.[6]
Excellent recent initiatives ultimately rely on political readiness to act. In Romania, for instance, the allocated budget of 23 million Euros for the next four years is supposed to ensure that at least 80% of estimated cases in Romania are diagnosed and treated. Yet the entire budget may be spent on a health system upgrade to start with, since treatment expenses only for their MDR TB population are much higher.[5,7-8]
Drugs cost 2000-6000 Euros per MDR TB patient, and estimated total costs for treatment in Romania are 50.000 Euros/ patient, as health care will probably not be as costly as in Western Europe.[6] Treating 1300 drug resistant TB cases costs Romania at least 65 million Euros a year. Apart from this financial investment, 30 physicians with MDR TB expertise are required based on a minimum 6 days direct patient contact a year.

High incidence countries should be offered to transfer drug resistant TB patients to countries with a low incidence. By "exporting" these cases, the troubled countries will have the time and the finances to strengthen and eventually rebuild their health care systems to be able to detect and treat patients in the near future according to the recently published EU/EEA standards of TB care.[9] Training of doctors and nurses at European standards of care with exchange programs may take place at the same time. Especially in case of this fastidious disease, this is to provide a moral boost for the health care workers and it will lower stigma around drug resistant TB as MDR and XDR TB in these countries generally means developing chronic TB or dying. Changing stigma will help patients to come in an earlier phase of the disease, will prevent transmissions and will improve treatment adherence. Most important, it will give the institutions time to fortify their health care systems. Inability to implement the above measures will further increase the problem, making it practically insolvable.[8-9]
For low incidence countries such as The Netherlands this policy will ensure maintenance of familiarity to the various facets of TB **(chapter 2)** and will also facilitate European TB research at high standards.[10] A facilitating condition for this plan is that referral TB centers have effective and prudent administrative and personal protection measures in place.[3-4] Secondly, negative-pressure ventilation rooms are available.[4] Finally, the centers are used to language barriers as the major part of TB populations in most low incidence countries are immigrants.[3]

Latent tuberculosis infections

Currently, multiple indications for biologic agents – particularly for anti-TNF α agents- are under investigation. As blockage of TNF α induces a higher risk of reactivation of TB, screening for LTBI becomes more and more important (***chapter 3***).[11-12] Similarly, in other parts of the world, especially where HIV is more prevalent, detection of TB latency is becoming increasingly important as recent studies showed again that isoniazid preventive therapy reduces the risk of tuberculosis or death by 44-58%, mainly in patients with positive TST results.[13-14] For these purposes we need a gold standard for LTBI diagnosis. Both tuberculin skin testing and interferon gamma release assays (IGRA) have limitations detecting LTBI.[12,15-18] Furthermore, a positive TST or IGRA, cannot predict reactivation. Future research should focus on predictive tests for TB reactivation.

On the other hand, updated national and European guidelines of LTBI screening in relation to the use of anti-TNF α blocking agents need to be evaluated meticulously in the coming years. To distinguish which screening test has the best positive predictive value and which is the best treatment regimen, a multi-centre and -country study is needed. A large amount of patients using biologic agents are required to study method and outcome of screening, prophylactic treatment prescribed, treatment adherence and reactivation of TB during the use of biologic agents. Local hospital TB coordinators and/or research nurses may have a central role in data collection and follow up in such a study.

Microbiology of M. bovis

As was shown in our *M. bovis* study (***chapter 3***), 17% of all patients did not receive treatment in accordance with international guidelines. This was accounted for by the treating physicians' unawareness of the fact that *M. bovis* is always resistant to pyrazinamide. A delay in susceptibility testing (DST) of *M. bovis*, explained by the slow growth of this subspecies on Löwenstein Jensen medium, ultimately led to incorrect therapy schemes. The majority of the patients in our study were elderly native Dutch individuals in whom we often fear increased toxicity or leave ethambutol from the start considering the possibility for drug resistant TB negligible.

In a recent epidemiological study conducted in England, primary resistance in *M. bovis* was found.[19] Studying our data again we also observed that six percent of our cases were resistant to isoniazid, two were MDR cases and two patients had a poly resistance (isoniazid and streptomycin). All of them occurred in immigrants from less affluent countries. There are several future means to improve the diagnosis and treatment of *M. bovis* infections (***chapter 3***). First, we need to study the reason of slow growth of *M. bovis* on conventional media and how to accelerate this process. Second, laboratory policy should be steered towards providing preliminary DST results from molecular studies in case of *M. bovis*. Third, TB coordinators ought to be educated to change treatment schemes when *M. bovis* is encountered. More specifically, clinicians have to stop using pyrazinamide and prolong treatment to nine months. In this way providing preliminary molecular drug susceptibility tests and education will prevent suboptimal treatment and acquired drug resistance in case of isoniazide resistance, especially in case of drug toxicities.

Active case finding

Tuberculosis case detection rates still remains low in large parts of the world. With the emphasis on passive case finding strategies, as propagated by the World Health Organization (WHO) for a long time, we will continue to underestimate the burden of the disease. This urges for a change (***chapter 4***).[4,20-21]

Active case finding in school children, teachers and army recruits, has been employed in The Netherlands from the beginning of the previous century and was abandoned around the eighties. Since 1970 active case finding of immigrants from Turkey and Morocco with chest X-ray (CXR) was employed again. The extension to high risk groups, with tuberculin skin testing (TST) and CXR, has been adopted since 1995 in The Netherlands. To distinguish between the exact effects of active case finding and the improved socio-economic welfare along with effective drug treatment remains difficult.

We have a well functioning TB control system and excellent treatment success rates.[22] It speaks for itself that our country provides many ingredients for success although studies from remote areas in Africa also show that active case finding is possible and worthwhile.[22-26] Referring to The Lancet, its article 'Time to act' highlights the inappropriateness of the ongoing debate on cost (in)effectiveness of active case finding strategies.[4,24,27]

Active case finding will allow for more early diagnoses at the expense of bacteriological confirmations. However, it boosts case detection rate and early treatments, preventing further transmissions, morbidity and mortality.[21] Obviously the extent of drug resistant mycobacterial strains in high incidence countries is scaled differently from ours and there is a risk of misdiagnosis[28] and insufficient treatment. But the majority of these countries still not have any possibility to determine the strain or perform DST. Upscaling the laboratories worldwide is very necessary[29] but time consuming and in the meantime we should try to diminish the expanding problem and try to reach the most vulnerable patient groups.

Active case finding relies on certain conditions. First of all enlargement of public awareness and education level of latent and active TB. Nowadays, a large part of the world has access to internet via computers and smartphones.

Via these media, advertisers constantly exhaust the public with commercials and it resulted quite easy to organize a wild but spontaneous "sweet-sixteen birthday party", in a remote Dutch village via Facebook recently. To reach the world, we need these popular media to get to people even in the remotest areas. I hereby announce an award for the most creative spreading initiative!

Another important issue is changing the current worldwide policy of 6 months isoniazid prophylactic treatment to an equally effective 3 month regimen of both isoniazid and rifampicin.[15-16,30-31] Shortened and amplified schemes in fixed dose combinations may solve important problems encountered with current prophylaxis, i.e. problems of adherence, isoniazid resistancy and acquiring drug resistance in case of treating active TB with mono-therapy. To screen contacts and risk groups, countries need solid public TB health care systems, ideally integrated into their HIV programs, and sufficient personnel.

The currently available trucks equipped as 'mobile-one-stop-shops' for TB and HIV diagnosis are excellent initiatives enormously facilitating the implementation of active case finding in remote areas.[20,24-26,32-33]

TDM at large scale

In an *in vitro* model, it was recently shown that treatment adherence, is not predicting treatment outcome, as was thought frequently. From this hollow-fiber model we understand now that pharmacokinetic (PK) variability is crucial in acquiring drug resistance and treatment failure.[34-35] In our study among TB patients in Dutch referral centers we observed large interindividual variability in pharmacokinetics **(*chapter 6*)**.
Furthermore, we observed low drug exposures in patients with treatment failures and relapse TB **(*chapter 7*)**.[36] Increasing the dosages of first line TB drugs led to culture conversions, clinical improvement and no further relapses. Higher dosages did not lead to the increase of adverse effects. For this reason, TDM should be central in patient care and drug trials. Very few centers in the world are currently applying TDM in patient care. To implement TDM at large scale, patient burden, especially for children, and cost is considered too high. The familiarity with the indication and usefulness of TDM of physicians in clinical TB care is not very large.
The first condition to implement TDM at large scale is the availability of accurate and precise analytical methods for bio-analysis of TB drugs. These methods should be validated and every assay should be accompanied by measurement of internal quality control (QC) samples with known drug concentrations. Furthermore an external (inter-laboratory) QC program, or proficiency testing program, should be available. Pharmacists involved in the two Dutch TB referral centers recently initiated such a program.[37] The QC program sends samples to laboratories with blinded concentrations of TB drugs to analyze. The program alerts the laboratory in case of detected errors to enable them to improve their assays or procedures if applicable.

Secondly, we should study simpler, less invasive and less time consuming methods for TDM. Intensive venous sampling **(*chapter 6 and 8*)** goes along with a high patient burden and high costs due to labor intensive sample extraction and processing. In our patient care service, we sample at 2h, 4h and 6h post dose to 'catch' the peak plasma concentrations (C_{max}) of TB drugs. Reference values for these peak plasma concentrations are available.[38]
This strategy is also applied in TB care in the USA.[38] An alternative strategy is to estimate the total exposure of TB drugs, the area under the concentration versus time curve (AUC), rather than C_{max}. In **(*chapter 6*)** we have derived the optimal sampling times that enable estimation of the AUC for some TB drugs. This resulted in limited sampling formulas with fixed sampling times. Alternatively, individual exposure can be estimated by combining measured concentrations at any (non-fixed) sampling time point with population pharmacokinetic parameters. Therefore, maximum a posteriori probability Bayesian fitting should be used.[39]
Less invasive methods imply less patient burden and may be less labor intensive. Especially for children, it would be advantageous to use saliva instead of plasma, as saliva can be collected by non-invasive techniques. Until now, only few studies have evaluated the correlation between salivary and plasma concentrations of TB drugs,[40-41] but more studies are currently conducted . The dried blood spot (DBS) technique, with blood from a simple finger prick on filter paper, already an established method for measurement of peak levels and exposure of several drugs (such as phenytoin, tobramycin, carbamazepine), seems promising in TB.

In the mentioned examples a cost-benefit discussion was never evoked, unlike in TB.[42] With ease, DBS recently found its way to patient care in home based intravenous antibiotics treatments with measurements of tobramycin levels in our Cystic Fibrosis Centre. The patients send the DBS from their homes to the hospital per postal services.
The first laboratory studies in DBS of first- and second-line TB drugs were recently published.[43-45] The accuracy of the method still may be affected by many factors, including paper type, blood characteristics, analysis method and the characteristics of drugs themselves. Clinical validation of this method is required for the interpretation of DBS concentrations versus serum or plasma concentrations. Pharmacogenetics can also be evaluated via this technique. DBS, which seems promising to perform TDM in TB treatment at large scale in less equipped areas, is currently awaiting validation studies.

TDM practice will be continued to individualize TB treatment in our TB referral centre (***chapter 7***). PK studies in fixed dose regimens will be evaluated in the near future in Dekkerswald and we will continue to perform PK studies abroad (in Tanzania and South Africa) as conducted in the last few years from our centre. A pharmacokinetic study, planned to be undertaken in Paraguayan native Indians, will compare conventional two time points venous sampling with DBS to evaluate exposure. Although no PK data are available from this geographic region, we do have data from Asia, Africa and Europe. An earlier study in Paraguay has shown that most native Indians from Paraguay are superstitious about blood extractions and therefore are more difficult to include in trials.[46] We will evaluate the correlation of the dried blood spots with conventional sampling, the exposure of native Paraguayan Indians to first line TB drugs and the patient burden. Likewise, children will be studied in the same hospital in Paraguay.

NTM research

The important interaction between rifampicin and clarithromycin leading to very low serum concentrations of clarithromycin demands re-evaluation of dosing schemes in NTM infections (***chapter 8***). Low plasma concentrations in TB treatment can lead to acquired drug resistance as was shown with the hollow-fiber model.[34-35,42] In a study testing drug susceptibility of NTM isolates, acquired clarithromycin resistance occurred in 22/237 (9%) of cases with serial isolates. It either occurred during therapy or was discovered when the patient relapsed.[47] Outcome of treatment for NTM is often disappointing. The interaction of rifampicin with macrolides is one of the possible reasons.

It is not clear whether this interaction plays a role in the observed discrepancies of the influence of macrolides on the outcome of treatment between American studies and the studies from the British Thoracic Society in 2008.[48-49] Increasing the dose of clarithromycin to compensate for the effect of rifampicin and to improve clarithromycin plasma concentrations should be evaluated in PK studies. The use of rifabutin instead of rifampicin might overcome this important interaction and a study is needed to evaluate the pharmacokinetics and tolerability in an HIV negative population. Azithromycin, a newer and better tolerated macrolide in a once-daily dose, is increasingly used in NTM treatment. In our study the effect of rifampicin on exposure to this macrolide was less pronounced.

The efficacy of azithromycin has never been studied in a randomized trial for NTM infections. For a randomized controlled trial that compares the efficacy of clarithromycin versus azithromycin or a treatment scheme without macrolides, large homogenous patient groups need to be studied to prove superiority. To include a large homogenous group of patients with NTM disease is very challenging. Hollow-fiber models might serve as surrogate markers for response and proof of concept. The *in vivo* response to treatment in clinical trials may prove to be a lot more capricious however.

The current thesis provides building blocks to fortify the defense against TB. The foundation is formed by practical recommendations for future Dutch (hospital) TB care, a possible short term solution for the increasing problems with drug resistant TB in the EU/EEA region and a call for adaption of active case finding policies for better global TB control. These recommendations evolved from epidemiologic, diagnostic and pharmacological studies in our relatively small scale TB population. Yet especially pharmacological PK studies do not necessarily need large numbers and can be done in centers as ours.
The currently conducted pharmacological (international) studies are important and innovative and may lead to changes in TB treatment and good clinical practice. Intervention studies in TB treatment, as for example the phase II High Dosage of Rifampicin Study from our centre performed in Moshi, Tanzania, are more pioneering and might become revolutionary in the near future. Promising building blocks will be added to the foundation in the years to come- but the cornerstones for improving TB care have been laid.

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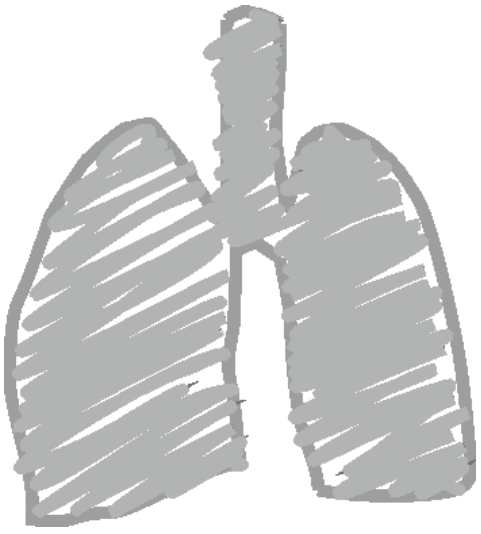
Achtergrond

Tuberculose (TB) teistert de mens al sinds de oudheid. Tekenen van deze ziekte worden gezien in graven van de farao's, waar mummies tekenen vertonen van "werveltuberculose" met invaliderende en mutilerende krommingen in de wervelkolom. Hoewel we sinds de jaren zestig van de vorige eeuw over een effectieve behandeling voor TB beschikken, is het geen enkel land in de wereld tot nu toe gelukt deze ziekte helemaal uit te roeien.
TB wordt veroorzaakt door bacteriën van het *Mycobacterium tuberculosis* complex. Dit complex bestaat uit verschillende subtypes die allen de ziekte TB veroorzaken. Hoewel de wereldwijde incidentie en sterfte nog steeds afneemt, worden ieder jaar nog zo'n 9 miljoen nieuwe TB patiënten geregistreerd met jaarlijks 1,4 miljoen doden. De incidentie van TB varieert enorm van 5 : 100.000 in Scandinavische, sommige Europese landen en de Verenigde Staten tot meer dan 300 : 100.000 in Zuid-Afrika.

Non-tuberculeuze mycobacteriën (NTM) worden de laatste jaren steeds vaker herkend als echte ziekteverwekkers. Ze zijn de verwekkers van een ziekte die lijkt op TB met als verschil dat het een niet-overdraagbare ziekte betreft en er nu sprake is van een stijgende incidentie, vooral in landen waar de TB incidentie afneemt. Deze stijging wordt veroorzaakt door verschillende factoren, waaronder verbeterde detectiemethoden, een groter bewustzijn van artsen, de toegenomen leeftijd van patiënten, de toegenomen incidentie van COPD, het wijdverbreide gebruik van immuunmodulerende geneesmiddelen en mogelijk een toegenomen blootstelling van het milieu aan deze bacteriën.
Dit proefschrift geeft een overzicht van epidemiologische, diagnostische en farmacologische studies vanuit Dekkerswald, een tertiair TB referentiecentrum voor patiënten met TB en NTM-infecties. De studies in dit proefschrift gaan over mycobacteriële infecties; over TB en één hoofdstuk over NTM-infecties.

Nederlandse samenvatting



Chapter 10

Introductie

De inleiding van het proefschrift (***hoofdstuk 1***) geeft een overzicht van de historie, de ontstaanswijze, de diagnosestelling en behandeling van TB en NTM. Vervolgens zoomen we in op de farmacokinetiek van de TB behandeling om te kunnen begrijpen dat de TB behandeling vele mogelijke bedreigingen kent voor het falen van de therapie, het optreden van recidieven en het verwerven van resistentie tegen geneesmiddelen. Tot slot wordt een overzicht gegeven van de Nederlandse TB bestrijding, waarin de rol van de klinische TB consulenten en de TB referentiecentra wordt verduidelijkt.

Epidemiologische studies

Dit proefschrift begint met een beschrijvende studie (***hoofdstuk 2***) van TB patiënten in Dekkerswald in de periode 2000-2005. Het geeft inzicht in de demografische, klinische en sociale gegevens van de populatie van ons centrum. Een totaal van 166 patiënten, met 37 verschillende nationaliteiten, werden geanalyseerd. In 98% van de gevallen was er sprake van een tertiaire verwijzing. De meeste patiënten (68%) werden verwezen om klinische redenen. 32% werd verwezen om sociale redenen, zoals dakloosheid, drugsmisbruik en psychiatrische toestandsbeelden. Ernstige levertoxiciteit was de meest voorkomende klinische reden voor verwijzing, gevolgd door een trage respons op de behandeling, de noodzaak tot chirurgisch ingrijpen, een combinatie van HIV en TB, resistentie tegen 1e lijnsgeneesmiddelen en de noodzaak tot isolatie van de patiënt. 94% van de resistente stammen werden geïsoleerd bij patiënten van buitenlandse afkomst. Toxiciteit en bijwerkingen leidden vaak tot veranderingen in de behandeling (40%). Tien procent van de patiënten was seropositief voor HIV en velen van hen (82%) hadden last van bijwerkingen van de behandeling, vooral levertoxiciteit. Extrapulmonale TB werd significant vaker gediagnosticeerd in de buitenlandse patiëntengroep. De mediane opnameduur voor de totale populatie was 10,1 weken. Deze liet geen verschillen zien tussen klinische- of sociale redenen voor opname of land van herkomst. Vijfentachtig procent van de patiënten voltooide de behandeling, zes procent werden uit het oog verloren bij follow-up en de mortaliteit was vijf procent. We concludeerden dat de TB populatie van Dekkerswald een uitstekend slagingspercentage van de behandeling heeft, ondanks de complexiteit van de ziektebeelden en de diversiteit van deze groep patiënten. Gezien de toename van het aantal opnames in Dekkerswald sinds 2005 lijken we te voldoen aan een behoefte in Nederland. Na het beschikbaar komen van een effectieve behandeling vanaf de zestiger jaren waren sanatoria al gauw niet meer nodig op grote schaal. TB zal echter nooit volledig verdwijnen en het is daarom van belang dat er enkele gespecialiseerde instellingen actief blijven om deze specifieke expertise te behouden en zelfs verder uit te bouwen op het gebied van resistente TB. Het volgende hoofdstuk (***hoofdstuk 3***) is een retrospectieve analyse van alle *Mycobacterium bovis* TB in Nederland van 1993-2007. We startte deze analyse nadat we kort na elkaar drie patiënten in Dekkerswald opgenomen hadden met *M. bovis* TB. We analyseerden de gegevens van 231 *M. bovis* TB patiënten en bestudeerden klinische- en demografische gegevens, behandelschema's, behandeluitkomsten en risicofactoren. De ziekte was voornamelijk extrapulmonaal van aard (n = 136; 59%). Hoewel 95 patiënten een pulmonale bovis TB hadden traden er geen besmettingen op van persoon tot persoon, zoals bleek uit structurele DNA-analyse. Vrouwen hadden meer kans een lymfeklier TB te ontwikkelen (p <0,0001), mannen ontwikkelden vaker pulmonale ziekte (p <0,0001). De

diagnose werd juist gesteld, maar de vertraging in de determinatie van de stam leidde frequent tot inadequate behandeling (17% van de gevallen). Het aantal sterfgevallen door *M. bovis* (5%) was hoger dan die voor *M. tuberculosis* (2%). Uit deze studie werd geconcludeerd dat de prevalentie van *M. bovis* ziekte in Nederland (1,4%) vergelijkbaar is met die in andere landen waar ook controleprogramma's voor rundertuberculose werden ingesteld. Sexe verschillen in klinische kenmerken en sterftecijfers werden gevonden in ons cohort van patiënten. De ziekte wordt heden ten dage vooral aangetroffen bij oudere autochtone Nederlanders (60%) en immigranten uit Marokko (23%). Anti-TNF-α behandeling is een recente oorzaak van endogene reactiveringen waarbij *M. bovis* TB trager reactiveert dan *M. tuberculosis*.

Diagnostische onderzoeken

In ***hoofdstuk 4*** werd de reden onderzocht van een daling van de kweek bevestigde TB diagnoses (72 % in 2007 tot 66% in 2009) in Nederland. Een Mycobacteriële kweek is de gouden standaard voor de diagnose TB en is nodig om de gevoeligheid van de bacterie voor de medicatie te bepalen. In deze studie hebben we studie gemaakt van de validiteit van de registratie van kweekresultaten in het Nederlands Tuberculose Register (NTR). Daarnaast hebben we de patiënten karakteristieken van de niet met kweek bevestigde TB diagnoses bestudeerd en hebben we gekeken naar de juistheid van de diagnoses. We bestudeerden de gegevens van patiëntendossiers van niet kweek bevestigde TB patiënten in de dossiers bij de GGD-tuberculosebestrijding. In het geval van ontbrekende informatie zochten we contact met de behandelaar van het ziekenhuis of het laboratorium. De resultaten werden vergeleken met de gegevens van het NTR. Volgens de criteria van het Europees Centrum voor ziektepreventie en ziektebestrijding (ECDC) werden alle niet met kweek bevestigde TB gevallen ingedeeld als 'bevestigd', 'waarschijnlijk', 'mogelijk' en 'geen' TB. Volgens de ECDC classificatie werd uiteindelijk 56% van de 516 onderzochte patiënten geclassificeerd als 'mogelijk TB', 35% als 'waarschijnlijk TB', en slechts 6,8% als 'bevestigde TB'. Van de niet met kweek bevestigde gevallen was 36% een vroege diagnose in de screening van risicogroepen (of contact onderzoek). Deze vroege diagnoses werden voornamelijk gesteld door artsen van de GGD-tuberculosebestrijding en gaan soms ten koste van kweek bevestiging. Een totaal van 37% werd niet geheel correct geregistreerd in het NTR, maar slechts 2,5% was uiteindelijk kweek positief. Specialisten in het ziekenhuis moeten zich bewust worden van het belang van hun bijdrage aan de Nederlandse TB registratie voor surveillance. Aan de andere kant kan extra scholing van de GGD'en de kwaliteit van de registratie van het NTR nog verder verbeteren. Een andere studie die betrekking heeft op de diagnostiek (***hoofdstuk 5***) geeft inzicht in de patient karakteristieken en de tijd tot aan diagnose van spinale TB in Nederland van 2000-2011. We voerden een beschrijvende, retrospectieve studie uit met gegevens van het NTR en patiëntendossiers van de GGD tuberculosebestrijding. We onderzochten 274 patienten dossiers en vonden een mediaan diagnostisch traject van vijf maanden (variatie 0 tot 76 maanden). Geslacht én leeftijdsgroepen werden geassocieerd met significante verschillen in diagnostische vertraging (resp. man 4,5 maanden vs. vrouw 5,5 maanden, p = 0,004 en 0 tot 34 jaar 4,5 maanden, 35-64 jaar 5,75 maanden en> 65 jaar 5,0 maanden, p = 0,006) . Opvallend genoeg werd er geen verschil waargenomen tussen de oorsprong van patiënten, patiënten die risicofactoren voor TB hadden of zich presenteerden met neurologische symptomen. Typische TB symptomen bij presentatie (pijn in de rug, nachtzweten en gewichtsverlies) leidden verrassend genoeg tot een sterk

patiënten die risicofactoren voor TB hadden of zich presenteerden met neurologische symptomen. Typische TB symptomen bij presentatie (pijn in de rug, nachtzweten en gewichtsverlies) leidden verrassend genoeg tot een sterk toegenomen doctors' delay (typische symptomen 4,0 versus geen typische symptomen 2,0 maanden, p = 0,05). Om TB hoog in de differentiaal diagnose van dokters te houden moet bijscholing steeds opnieuw worden aangeboden aan huisartsen en klinisch specialisten.

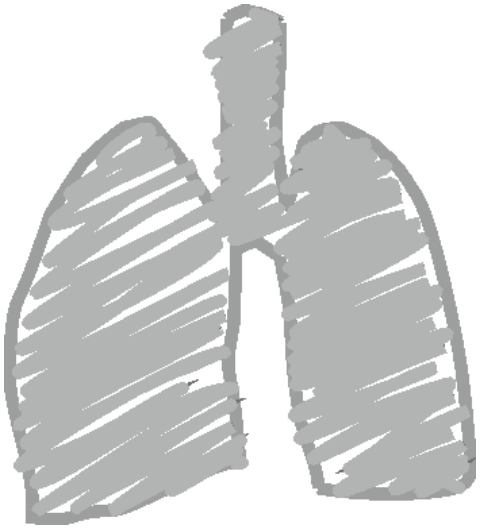
Farmacokinetiek studies

Om de resultaten van behandeling te optimaliseren is een beter begrip van de farmacokinetiek van TB medicatie noodzakelijk. Informatie over de precieze blootstelling aan een geneesmiddel lijkt van cruciaal belang om mechanismen als therapie-falen, het optreden van recidieven en de verwerving van resistentie beter te kunnen begrijpen. In plaats van het toepassen van alleen vaste doseerschema, kunnen de geneesmiddelconcentraties worden gemeten en kan de dosis op basis van de vastgestelde concentratie (Therapeutic Drug Monitoring, TDM) worden geïndividualiseerd. Echter, omdat de relatie van de totale blootstelling aan het geneesmiddel en het effect op de uitkomt van de behandeling nog niet goed bekend is, wordt TDM slechts op beperkte schaal toegepast. Bovendien zijn de belasting voor patiënt, de kosten en de hoeveelheid tijd gemoeid met het meten van de bloedspiegels ook nog belemmerende factoren. Dit geldt voor TDM binnen de patiëntenzorg als ook bij de evaluatie van nieuwe TB medicijnen in onderzoeksverband.

Hoofdstuk 6 is een beschrijvende farmacokinetische studie van de anti-TB medicijnen binnen een serie van opgenomen patiënten uit de twee Nederlandse TB referentiecentra. Eenenveertig kweek bevestigde TB patiënten participeerden in de studie. Bij hen werd een 24-uurs farmacokinetische curve afgenomen na het bereiken van 'steady state'. Grote inter-individuele variatie in de farmacokinetiek van de geneesmiddelen werd aangetoond en 32% bereikte niet de minimale referentie plasma piek concentratie voor rifampicine. De reeds eerder beschreven interactie van rifampicine en moxifloxacine werd ook in deze studie gevonden. We voerden een lineaire regressie analyse uit om te kunnen adviseren over de tijdstippen van bloedafname na het innemen van het medicijn die het beste correleren met de totale blootstelling aan het geneesmiddel. De tijdstippen 1, 4 en 6 uur na inname waren de beste tijdstippen. Beperking van het aantal bloedafnames van 11 naar 3 om de totale blootstelling te kunnen bepalen, zal TDM binnen routine patiëntenzorg of onderzoek vergemakkelijken. In het volgende hoofdstuk (***hoofdstuk 7***) bieden wij inzicht in de huidige TDM praktijk van Dekkerswald. TDM wordt gebruikt bij patiënten met een normaal gevoelige TB waarbij abnormale TB concentraties van het geneesmiddel worden vermoed (bijvoorbeeld in het geval van HIV-infectie, diabetes, gastro-intestinale chirurgie in het verleden, ondervoeding, of nier- of leverinsufficiëntie), wanneer er sprake is van een vertraagde sputumkweek conversie (dat wil zeggen na meer dan acht weken van de behandeling), en in het geval van een recidief tuberculose. We presenteren een korte klinische samenvatting van vier TB patiënten met een recidief TB om daarmee de methode van TDM en de waarde van geïndividualiseerde therapiedosering te illustreren.
In de vier gevallen resulteerde verhoogde doses van de eerste lijn TB middelen, in het bijzonder rifampicine, in adequate plasma piekconcentraties en uiteindelijk een goede klinische respons op de behandeling. Er traden geen bijwerkingen op. Zoals reeds eerder vermeld is TB de belangrijkste pijler van dit proefschrift, maar NTM infecties zijn een

entiteit die niet langer kunnen worden genegeerd. Last but not least, verschuiven we ons focus daarom van TB naar een (farmacokinetische) studie bij NTM in ***hoofdstuk 8*** van dit proefschrift. In het algemeen zijn behandelresultaten voor NTM-infecties slecht, zelfs wanneer de aanbevolen doseringen van geneesmiddelen worden toegepast. In NTM populaties zijn zeer weinig farmacokinetiek studies uitgevoerd. Wij voerden een uitgebreide PK studie uit en hebben de plasma farmacokinetiek beoordeeld van rifampicine, ethambutol, claritromycine, azitromycine, isoniazide en moxifloxacine en de actieve metabolieten in een reeks van 14 patiënten met klinisch relevante NTM longziekten. We vergeleken onze resultaten met de beschikbare literatuurgegevens.
We leerden dat blootstelling aan claritromycine in combinatie met rifampicine zeer laag was en de gemiddelde medicijn-metaboliet ratios ook laag waren. Dit komt waarschijnlijk door de inductie van metabole enzymen door rifampicine. De totale blootstelling aan rifampicine was relatief hoog in vergelijking tot een TB populatie. Alle patiënten hadden een rifampicine C_{max} waarde boven de referentiewaarden van 8 mg / L. Ook alle ethambutol C_{max}-waarden lagen binnen de referentiewaarden (2 en 6 mg / L). Uit deze studie hebben we geconcludeerd dat de relevante interactie tussen claritromycine en rifampicine vraagt om een heroverweging van de doseringsstrategieën in NTM longziekten, omdat suboptimale blootstelling aan geneesmiddelen zouden kunnen bijdragen aan de matige respons op therapie. ***Hoofdstuk 9*** geeft een samenvatting van de studies en de implicaties voor verdere patiëntenzorg en onderzoek. Uit de tabel in hoofdstuk 9 worden de implicaties van onze studies voor toekomstige TB zorg en preventie verduidelijkt. De zorg voor TB patiënten in een welvarend land als Nederland biedt alle noodzakelijke middelen. Daarentegen blijft TB nog steeds de onoverwinnelijke ziekte die het al is sinds de oudheid. TB referentiecentra en klinische TB consulenten voorzien op dit moment in een behoefte in Nederland en zullen nodig blijven in de toekomst. Ziekenhuis TB coördinatoren zullen een belangrijke rol gaan spelen in de toekomst. Hun kennis en expertise moet gegarandeerd zijn daar ze de spin in het web zullen zijn in screening, preventie en kwaliteitsbewaking van de TB zorg in de ziekenhuizen. Het TB surveillance register zal nog verder kunnen verbeteren door een meer actieve betrokkenheid van specialisten in het ziekenhuis.
De kennis van de Nederlandse TB centra zouden gebruikt kunnen worden om probleemgebieden binnen Europese regio (EU/EEA) te gaan helpen bij de behandeling van grote aantallen patiënten met resistente TB. Anderzijds hebben onze studies ook voer voor verder tuberculose onderzoek gegeven. Er blijft een grote behoefte bestaan aan een gouden standaard voor latente TB infecties (LTBI). Ook is het belangrijk de nationale en Europese richtlijnen voor screening en behandeling van patiënten met LTBI in de toekomst te evalueren. Voor de directe patiëntenzorg is het versnellen van de groei van *M.bovis TB* stammen voor determinatie en gevoeligheidsbepalingen belangrijk.
Tot slot, de huidige manier van therapeutic drug monitoring, de spiegel bepalingen van TB medicijnen, rechtvaardigt eenvoudigere, minder invasieve en minder tijdrovende methoden. Dit proefschrift heeft een aantal bouwstenen in de strijd tegen TB opgeleverd; praktische aanbevelingen die volgen uit epidemiologische, diagnostische en farmacokinetiek studies in een Nederlandse TB populatie. Nieuwe, minder invasieve technieken in de bloedspiegelbepalingen van TB medicatie kunnen gaan leiden tot veranderingen in onze huidige TB behandeling en ' good clinical practice'. Van de kennis die we hebben opgedaan kunnen wetenschappers die zich bezighouden met medicijnstudies in de toekomst profiteren.

List of publications



Chapter 11

List of publications

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11. Magis-Escurra C, van den Boogaard J, Ijdema D, Boeree M, Aarnoutse R. Therapeutic drug monitoring in the treatment of tuberculosis patients. Pulmonary pharmacology & Therapeutics. 2012;25(1):83-6

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Dr. Rob Aarnoutse, copromotor, dank je wel voor je commentaren, aanwijzingen en correcties die je maakte op de stukken. Ik denk met veel plezier terug aan de momenten die we samen doorbrachten pratend over farmacokinetiek en niet te vergeten de statistiek. Prachtig om te zien hoe secuur je bent en je nooit iets laat passeren zonder dat dit persoonlijk nog door jou is nagerekend. Mooi om te ervaren hoe een apotheker daarin verschilt van een clinicus. Zonder jouw fantastische hulp was het me zeker niet gelukt het proefschrift uiteindelijk af te ronden.

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Dr. Hanneke Nijland, met veel enthousiasme hebben we (alweer) jaren geleden samengewerkt aan de farmacokinetiek studie van de patiënten in Dekkerswald en Beatrixoord. Mooi dat we deze studie hebben kunnen uitbreiden met de belangrijke limited sampling strategie.

Ook dank aan Angela Colbers die me geholpen heeft bij de farmacokinetiek berekeningen in het Winnonlin programma dat er heel eenvoudig uit ziet als je ernaast zit maar als je het zelf moet doen toch wel veel oefening vereist .

Marieke Dekker, wat geweldig dat je mijn paranimf kunt zijn vandaag. Jouw hulp bij de tot standkoming van mijn proefschrift was geweldig. Door onze gezamenlijke gesprekken en later de e-mails, toen je naar Tanzania vertrok, kon ik zaken beter relativeren en af en toe heerlijk stoom bij je afblazen. Ik heb een enorme bewondering voor je hoe je met je grote gezin nu voor een paar jaar in Afrika woont en werkt.

Flora Verbrugge, fijn dat je mijn "soort bruidsmeisje" wil zijn op deze dag. Voor jou als artistiek leider van een van de weinige nog gesubsidieerde theatergezelschappen in Nederland moet de ceremonie van deze dag een groot feest zijn. Leuk dat je er bij bent en me herinnert aan een mooie tijd in Enschede die we samen doorbrachten op de Onlandhorst maar vooral ook aan onze nog steeds voortdurende gezamenlijke traditie tijdens de donkere dagen.

Collega's van Dekkerswald; Monique Reijers, Jeanine Antons, Johan Molema, Jolanda van Haren, Wouter Hoefsloot en natuurlijk Inge de Guchteneire, jullie hebben me in het laatste jaar enorm gefaciliteerd zodat ik rustig thuis kon gaan werken aan het boekje. Veel dank voor jullie steun en interesse.

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Manuel Arbo, thanks for the time I have spent in Paraguay as a resident in infectious and pulmonary diseases. You have been an excellent role model for me all those years and you really made me enthusiastic for infectious diseases. Thanks for your efforts during my stay in Paraguay in 2003 in which we performed our study.

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Tomas, Camila en Susana; jullie zijn drie schatten die heel groot aan ´t worden zijn. Dank voor de rust en de tijd die jullie me gunden om thuis te kunnen werken aan "het boekje". Jullie zorgen voor veel beweging in ons huis maar zijn stuk voor stuk prachtige kinderen waar ik heel erg trots op ben.

Curriculum Vitae

Cécile Magis is geboren op 27 augustus 1967 in Helmond. Na het behalen van het VWO diploma aan het Carolus Borromeus College, begon ze in 1986 eerst aan een studie psychologie en werd ze in 1987 ingeloot voor de studie geneeskunde aan de Universiteit van Utrecht. Tijdens de studie deed ze gedurende 4 maanden een keuze co-schap in het academisch ziekenhuis voor tropische infectieziekten (*Lacimet*) in Asunción, Paraguay.

Het artsexamen werd een aantal maanden later behaald in 1995. In Januari 1996 vertrok ze naar Paraguay om daar een jaar als arts assistent infectieziekten (opleider: Manuel Arbo Seitz) in *Lacimet* te werken. In 1997 besloot ze nog een jaar in Paraguay te blijven en werkte ze als arts assistent longziekten en tuberculose (opleider: Gilberto Chaparro Abente) in het academisch ziekenhuis *Juan Max Boettner/Instituto Enfermedades Respiratorias y Ambientales; INERAM*. Tijdens deze twee jaar werkte ze, naast het assistentschap, een middag per week als arts in de staatsgevangenis in Asunción (*'Tacumbu'*) voor een groep AIDS patiënten met een subsidie van de Europese Unie. In de avonduren was ze tweemaal per week verbonden als algemeen arts aan een instituut voor verstandelijk en lichamelijk gehandicapten *'el Cottolengo'* in Mariano Roque Alonso, een dorp net buiten Asunción.

In Augustus 1998 startte ze als arts assistent bij de afdeling longziekten in het Medisch Spectrum Twente te Enschede. Vanaf 1999 startte ze daar de opleiding tot longarts (opleider: Dr. J. Klein). Tijdens haar opleiding verrichtte ze gedurende twee maanden tuberculose onderzoek in het *INERAM* in Paraguay. Sinds April 2005 is haar opleiding tot longarts voltooid en is zij sindsdien verbonden aan de afdeling longziekten van het Radboud Universitair Medisch Centrum, gedetacheerd op het Universitair Centrum voor Chronische Ziekten Dekkerswald in Groesbeek. Als aandachtsgebieden heeft ze tuberculose, non-tuberculeuze mycobacteriële infecties, cystic fibrosis en bronchiectasieën. Sinds 2010 is ze tuberculose coördinator van het UMC Nijmegen en nationaal consulent klinische tuberculose van de KNCV. In 2013 is ze gestart als expert namens de KNCV in een internationaal panel binnen een scholingsprogramma via internet over drug resistente TB. Na haar promotieonderzoek zal ze haar onderzoeksactiviteiten op het gebied van tuberculose en non-tuberculeuze mycobacterieën voortzetten waarvoor ze in de zomer van 2012 de eerste onderzoeksplannen reeds heeft besproken met de collega's in Paraguay.

Ze is gehuwd met Hugo Escurra Ibañez. Samen hebben ze drie kinderen, Tomas (1998), Camila (1999) en Susana (2003).